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NORMAL AND MALIGNANT CELLS[1]

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THIS evening I propose to discuss the proposition that malignant cells are permanently altered cells. They are new types or species of cells that arise in the body from normal, usually adult, cells which have been altered by environmental influences or agents of one sort or another. The alterations appear to be irreversible in the body and in vitro under the ordinary conditions in which the cells live and multiply. It is conceivable that just as normal cells can be converted into malignant cells by an extraordinary environment, so perhaps malignant cells of one type may be changed into other types (of malignant cells) or reconverted into normal cells by a different environment. The pathologists speak of tumors changing. This may be explained either by an alteration of the malignant cell or by the gradual overgrowth of one special type among two or more types that were originally present. After malignant cells are once established they multiply independently of the special environment or agents which produced them, as is amply illustrated by the growth of metastases, by serial transplantations from animal to animal and by serial in vitro cultures. They can multiply indefinitely *in vivo* and *in vitro*. Many are cytologically different, that is visibly different from normal cells of the type from which they arose.

Speculations on the origin of malignant tumors or neoplasms have extended over a long period of years. Advocates, led astray by their own pet theories, have fought for this and that idea. In my student days there came to Baltimore pleaders for the protozoan origin of cancer, and following this a distinguished protozoologist worked out the life history of the

[1] Presidential address, read before the American Association of Anatomists, at the St. Louis meeting, April 19, 1935.

cancer-producing protozoan. Peculiar bodies seen in some carcinoma cells led to this view, but since they are not present in various other types of cancer the idea soon died a natural death.

Bacteria have in turn been pushed to the front as the causative agents of cancer. Bacteria like protozoa have been eliminated from the race, although there are still a few who believe that they can produce malignant tumors. The work of Irwin Smith on the tumors in plants produced by the *bacterium tumefaciens* gave an added impetus some years ago to the idea, when it was found that remarkable tumors could be produced in animals with a similar organism recovered from a human breast carcinoma. No evidence has appeared that the cells of such growths can multiply independently of the organism and until such evidence is conclusively demonstrated these tumors should not be classified with malignant ones.

There are still investigators who maintain that tumors are probably due to viruses. They state that viruses may produce every type of cell change from pure necrosis to almost pure cell proliferation. They point to a series of virus epithelial tumors ending with non-filterable ones and malignant epithelioma and to a series of connective tissue tumors, beginning with ones produced by filterable viruses and ending with non-filterable fowl and mammalian sarcomas, and ask where a line is to be drawn between infectious tumors and so-called true tumors or the ones that we would say are not dependent on the presence of any infectious agent for their origin and growth. Many attempts have been made to recover viruses from true mammalian tumors and to reproduce the tumors with filtrates but so far without success, although claims to the contrary have frequently been made. The participation of viruses in the production of what we might term true tumors is not easy to eliminate, but a provisional line can be drawn between them. The virus tumors can be produced by cell-free filtrates but not the malignant ones. Some day it may be shown that some of the tumors now considered malignant are in the virus class. Another profound difficulty exists in the uncertainty as to the very nature of viruses. Are they living organisms or merely chemical substances? But even with this uncertainty we can separate the true tumors from the turmoil by depending on whether cell-free material from them will or will not produce the tumors. This leaves the famous Rous chicken sarcoma on the virus side, where it probably belongs.

Although one might explain the multiplication of malignant cells by the presence of a virus which increases with cell increase it is difficult to understand, if a virus is always present, why after tumors are once started no additional cells of the host are converted into malignant ones. In the growth of metastases, from epithelial tumors, in the spleen, the lungs and other organs one can see plainly that they are composed of descendants of cells from the original tumor; they stand out in marked contrast to the surrounding tissues. The same is true when tumors are transplanted from animal to animal. One can back out of the situation by assuming that the virus is so intimately attached to or secured within the cells that it can not get away to neighboring cells while it can readily be transmitted to daughter cells where it multiplies for the next generation of cells. Most tumors have many dying and dead cells offering a chance for the release of the virus, but this does not seem to result in changing neighboring normal cells into malignant ones. Having somewhat arbitrarily ruled out all the virus tumors from our class of malignant ones we will proceed to other tumor-producing agents.

One of the most, if not the most, prolific sources of induced animal sarcomas are the tapeworm cysts of the liver resulting from the feeding of tapeworm eggs of the cat to rats. At the Institute of Cancer Research, New York, over 4,300 such tumors have been produced in this way. The larvae pass from the stomach to the liver and induce cyst formation about them. In the walls of such cysts there frequently develop after many months typical sarcomata of one sort or another. There has been considerable speculation in the minds of people who have had anything to do with tumors as to the causative factors involved. Long-continued irritation, the favorite factor of the clinicians, the possibility of a virus being carried along with the larvae and of chemical products of the latter have all been considered without arriving at a definite answer. From none of these tumors has a filterable virus been obtained that will reproduce the tumor. A number of these tumors have been transplanted from animal to animal for considerable periods of time, and we have had the privilege of studying several. One of them has been carried for over 4 years in vitro. They fall readily into the class of malignant tumors.

The occurrence of certain occupational cancers led two Japanese workers to experiment with tar. They succeeded in producing cancers on the ears of mice after repeated applications over a long period of time. This has been many times repeated and led to testing out of purified products from tar. During the past few years, some English workers have succeeded in producing both carcinoma and sarcoma in mice and rats with such products, namely, 1:2:5:6 dibenzanthracene and related substances.

These chemically pure substances produce typical malignant tumors in a relatively short time, six months or less with the dibenzanthracene and in a considerably shorter time with methylcolanthrene. The sarcomata were produced by injecting subcutaneously under

strictly aseptic precautions very small amounts of dibenzanthracene dissolved in lard. These experiments would seem to eliminate the direct participation of living agents of any sort as the cause of the tumors and also to eliminate the old idea that true tumors are produced by the uncontrolled multiplication of normal cells induced by the presence in, on or near the cells of a living agent. It is interesting to note that these recently discovered tumor-producing substances are chemically related to the oestrous-producing hormones. So now it is quite stylish to work on the hormonal origin of tumors. With the new group of tumors produced by chemically pure substances 1:2:5:6 dibenzanthracene there is a fine opportunity to test out the filtrates. My guess is that such tumors will not give filtrates with either virus or chemical agents, which can reproduce the tumor especially after they have been transferred for a few times from animal to animal.

Having eliminated for the time being at least the idea that malignancy is due to living agents of any sort we must also eliminate the continued presence of some chemical agent that might be responsible for the peculiar properties of malignant cells such as (1) the uncontrolled growth in the body, (2) the transplantability to other animals of the same strain or species, (3) the peculiar cytological characters which serve to distinguish them from normal cells and (4) the maintenance in vivo and in vitro of these characteristics for generation after generation and year after year.

It is conceivable that the continued presence in, on or near normal cells of some special chemical agent might be responsible for the peculiar properties which are assigned to malignant cells. Let us suppose that four fibroblasts are converted into malignant cells at the site of injection of 4 mgm of dibenzanthracene, which is enough to induce a tumor. In a few weeks a tumor 40 mm in diameter will result with something like 4 billion cells. There will then be about one billionth of a milligram per cell to keep it in line. Suppose a number of metastases have developed in the body, since they consist of descendants of the original tumor cells, the allowance of dibenzanthracene per cell may then be not one billionth of a milligram but one two or one three billionth of a milligram. One may assume that that is enough of the agent to keep the malignant cells malignant. We are so accustomed to talking in billions these days that billionths may not seem so small.

It seems improbable, however, that such an agent could remain present in sufficient quantities to continue potent during the many cell divisions involved either in vivo or in vitro when cells are transplanted from animal to animal or culture to culture over a period of years, unless there was a continued addition to the original amount of the chemical agents which started the cells off in their new line at the time of the origin of the tumor. In the course of a month rat sarcomas often attain diameters of 40 mm or more. From such a tumor 100 rats could be inoculated. At the end of another month 10,000 could be inoculated. At the end of a year the astronomical number of 10^{24} rats would be running around, each with 4 billion tumor cells. If the chemical agent were as simple as water there would be only about one half a molecule per cell. So we can dismiss the notion that the presence of some of the original chemical agent keeps the cells malignant, or keeps otherwise normal cells in this condition.

It is possible that the chemical agent could be supplied by the host to keep the cells malignant during their multiplication in the original animal in which the original tumor arose. It is rather improbable that animal after animal for generation after generation could supply the agent to keep the transplanted tumor cells going and next to impossible for such a chemical agent to be introduced into a long series of in vitro cultures automatically with the medium where for example rat tumor cells are cultivated in horse serum, chick embryo juice, chicken plasma and a saline solution.

There is another idea which we might dwell on for a moment, namely, that the original agent might stimulate the cells to produce more of that particular agent and thus maintain the malignant nature of the multiplying cells. This is visionary and would imply an alteration of the cell.

I have omitted the x-ray cancers and the various spontaneous malignant tumors of man and animals. The very designation of the latter as spontaneous indicates that we know nothing definite about their origin. The x-ray cancers presumably arise as a result of injury to the epithelial cells, the presence or supposed presence of a ubiquitous virus is difficult to rule out, but the probabilities are that they too arise from altered cells.

These considerations thus seem to eliminate the idea that malignant cells are merely normal cells under the continuous stimulation of some extraneous agent. The most plausible alternative concept is the one we are considering, namely, that malignant cells are permanently altered cells derived from normal cells.

We have little definite information as to the steps in the alteration. There are as already noted a number of known agents which can change normal into malignant cells. The known agents take weeks or months to produce malignant cells or to produce tumors that are palpable. This may indicate long-continued action and slow gradual change of one or a few cells or it may indicate that many cells are altered in various ways and that only rarely is the alteration of such a char-

acter as to produce a malignant cell. Dr. Mendelsohn, working in our laboratory, has found that fourteen-day tapeworm cysts of the liver show all sorts of abnormal mitotic figures similar to those found in tumors, yet palpable tumors do not appear until 8 to 22 months later. What relation these early abnormal mitoses bear to the origin of tumors is uncertain, but it shows that the cells are already being upset long before tumors appear.

Having succeeded in getting the malignant cell started as an independent type of cell let us consider some of the differences between normal and malignant cells.

One of the outstanding differences between normal and malignant cells is controlled versus uncontrolled multiplication in the body. All normal cells are under control of some sort. The nature of the control mechanism is obscure but fundamental for all multicellular organisms. It begins at the 2-cell stage and continues throughout life. So familiar are we with the manifestation of this law that deviations from it at once attract attention. The control is elastic, it permits of increase and decrease of organs and parts through use and disuse, disease and recovery, injury and healing and regeneration. The end result is a return or an attempt to return the part to the normal condition. The swellings that develop in some infections from induced cell multiplication are sometimes remarkably like malignant tumors, but when the infectious agents are overcome the tissues tend to return to normal.

Malignant cells, on the contrary, continue to multiply like parasites, frequently spread through the body and continue to increase in number until they kill the host. This uncontrolled multiplication of the malignant cells sets them apart from all normal cells. All tumors have this important characteristic of uncontrolled growth in common. Some very malignant ones grow and spread like wild fire and kill in a short time, others grow slowly and benign tumors may last for years and years as though their cells were almost under control. All gradations between these extremes are found. We are concerned more especially with the cells of malignant tumors, rather than with those of benign ones.

Malignant cells tend to grow in a disorderly manner, especially the more malignant ones, and produce structures with little resemblance to normal organized tissues. Their metabolism is altered. The production of normal secretions is debatable. The usual assumption is that they produce harmful rather than useful metabolic products. The increase in malignant cells thus can not be attributed to a demand by the rest of the body for their secretions as with normal ones.

There are, as you know, quite a number of transplantable rat and mouse tumors that have been carried for years by serial inoculations of small pieces of the tumor from animal to animal of the same species. Several tumors have been thus maintained for many years, ten, fifteen, twenty and even thirty years. The essential part of the technique is that living malignant cells shall be inoculated into animals of the same species or strain. There are some tumors that take in nearly 100 per cent. in almost any strain; others take in smaller percentages and still others take in only the particular strain in which they originated. Of the many spontaneous and induced tumors of rats and mice only a small percentage has been found to be transplantable. This is partly because some have not been tested or have been given only a perfunctory trial and partly because it often makes a very great difference as to whether or not inoculations were made into animals of the same strain and on the purity of the strains. Many of the failures may be attributed to the fact that the tumors tested originated in such mixed strains that no two animals had anything like the same genetic constitution. Recently a series of tumors were produced in five inbred strains of mice and one "stock" mixed strain with 1:2:5:6 dibenzanthracene by Andervont. Some mice of each strain developed tumors. There were about 100 per cent. "takes" when tumors were transplanted into animals of the same strain in which the tumor originated and 100 per cent. negative results when transplanted into the other strains. The subsequent serial transmission of these tumors to animals of the same strain were about 100 per cent. successful and 100 per cent. negative into animals of another strain. This illustrates the importance of dealing with pure inbred strains and may explain the negative results in the many attempts of serial transmission in mixed strains where no two animals have the same genetic constitution.

It is well known that in mammals most normal cells and tissues do not live long when transplanted to another animal of the same species. This curious individuality of each animal's tissues has received considerable attention from St. Louis' pathologist, Leo Loeb. The surgeons are well aware of this specificity from the universal failures with skin grafts from one individual to another. Attempts made to so cultivate skin epithelium that it will lose this individual specificity and thus be transplantable to any one have not been successful. The failures of ordinary normal tissue transplants from animal to animal and man to man are probably associated with differences in genetic constitution. No two animals of mixed colonies and certainly no two humans, except identical twins, have the same genetic constitution. When we turn for example to pure inbred strains of guinea pigs the success of cross transplantation of normal tissues increases with genetic identity as shown by Leo Loeb and his asso-

ciates. Brother to brother transplantations in an inbred guinea pig strain were nearly equal to autotransplantations. In inbred strains of rats, however, through brother and sister matings up to the forty-seventh generation, there seemed to be no distinct diminution in the reaction against transplants within the inbred family as compared with non-inbred ones.

On the whole, however, malignant cells are much more readily transplantable from animal to animal than normal ones, especially among animals of mixed genetic strains. This is unfortunate in view of the desirability of supplementing defective organs of one sort or another by the transplanting of normal organs or parts of normal organs into individuals with defective ones. Efforts are being made to train normal cells into transplantable ones. If they could be made slightly malignant or, perhaps better still, converted into benign ones in vitro the problem might be solved. At present we are still much in the dark as to why malignant cells are transplantable and normal cells not.

The behavior of malignant cells when cultivated over long periods of time in vitro is a strong argument for the idea that they are permanently altered cells. The malignant cells of a mouse adenocarcinoma have been carried in pure cultures for seven or eight years by Fischer without losing their malignancy. Carrel and Ebeling carried two rat sarcomas for 16 months with the same result. We have at present pure cultures of malignant cells from six different rat sarcomas that have been cultivated in vitro from one and a half to over 4 years. They have retained their essential cultural and cytological characteristics and their malignancy during the cultivation.

These sarcomas are all well-established transplantable tumors that had been carried on in rats for generation after generation for 3 to 20 years. Our studies on the behavior and cytology of the malignant cells from these tumors in simple hanging drop cultures began after the tumor had become well established in animals and were continued for some time before serial cultures were undertaken and were also carried on parallel with the serial cultures. The many series of cultures in simple hanging drops of chicken plasma, rat plasma and combinations of the two, with and without neutral red, enabled us to become quite familiar with the cultural and cytological characteristics of the malignant cells. Both the outgrowth patterns and the cytological characters of the cells are different from those of normal cells and from one another.

Attempts were made to cultivate a number of other transplantable rat and mouse tumors in serial cultures, only to have the cells die out on our hands after a few generations. I attribute the failures to lack of suitable media and imagine that some day it will be possible to cultivate almost every type of malignant cell indefinitely outside the body.

The six tumors which we have carried on in serial cultures comprise three round cell sarcomas, one spindle cell sarcoma, one rhabdo-myosarcoma and one polymorphous (mixed) cell sarcoma. The small pieces of tumor tissue with which the serial cultures were started contained many macrophages, monocytes, lymphocytes, fibroblasts and endothelial cells in addition to the malignant cells. Thus the primary cultures were mixed and sometimes contained all the above types of cells in the migratory zone. After a varying number of transfers we found without any special effort that the colonies contained only malignant cells with the possible exception of those from the polymorphous cell sarcoma. Various combinations of chicken plasma, dog plasma, human plasma, rat serum, human placental serum, horse serum, beef embryo juice, chick embryo juice and saline were used.

The colonies were carried in two types of cultures, the large sitting drops by Mrs. Gey and the roller tubes. In the former the cell colonies were replanted every four days into fresh clots without a supernatant nutrient fluid. The colonies in the roller test-tubes were replanted at varying intervals of 4 to 21 days, depending upon the condition of the colonies. The roller tube cultures have a thin clot of blood plasma lining the tube in which the colonies grow and a supernatant nutritive fluid which was changed every 4 days. From time to time simple hanging drop cultures were made from the large colonies for cytological examination. Rats were also inoculated every few months with one to several colonies to determine the malignancy. Up to the present the cells have maintained their essential cytological characters and malignancy. Some are not in as good condition as those taken directly from tumors, while others seem to show no ill effects from their prolonged life in vitro.

The inoculations into rats of the pure colonies of malignant cells have resulted in about the usual number of "takes" giving rise to typical tumors. Cultures from these tumors displayed the typical array of malignant cells, macrophages, monocytes, lymphocytes, fibroblasts, etc. The malignant cells were similar to those from tumors that have been carried on from animal to animal during the same period and the period preceding the serial culture experiments. The malignant cells, not in good condition, frequently produced typical tumors, and cultures from them revealed good healthy malignant cells, indicating their revival after inoculation into animals, and also that they were not seriously injured or modified.

In a few instances serial cultures were started from tumors produced by the inoculation of pure cultures.

These serial cultures gave in due course of time pure strains of malignant cells which in turn produced typical tumors. The tumors produced by the pure strains of cells are transplantable as were the progenitors of the tumors of the pure strains and some have been carried on from animal to animal for a number of generations.

The conclusive evidence that one has obtained the malignant cells is their recognition in cultures, their cultivation in pure strains, the production, on inoculation of the latter into animals of the same species or strain, of typical transplantable tumors and the recognition again of the malignant cells in cultures of such tumors. These conditions have been fulfilled with the above six rat sarcomas. The fact that the malignant cells have maintained, through all the vicissitudes of prolonged serial cultivation, their essential and recognizable cytological as well as their cultural characteristics which are peculiar for the malignant cells of each tumor and are different from normal cells speaks strongly for the idea that they are permanently altered cells that bred true.

Let us now turn to the most difficult task of all, namely, to give you in words an adequate idea of the cytological differences between normal and malignant cells and between the various malignant cells themselves. Our observations are based on the study in tissue cultures of the malignant cells of 27 rat and mouse tumors. Some have been studied again and again over a period of years, others in only a few or a single set of cultures.

Let us consider sarcoma cells with which I am most familiar. Supposedly they come from fibroblasts or other cells derived from the middle germ layer, and until we know better I shall compare some of the malignant sarcoma cells with fibroblasts. If I could lay before you a series of photographs which I have in Baltimore of living fibroblasts and of living sarcoma cells at a magnification of 1,000 diameters you would see at once that malignant cells are different from fibroblasts, and then you would begin to see that the sarcoma cells from each tumor were different from those of every other tumor. If I could put before you groups of photographs of a number of cells from each of the tumors you would see that the cells in each group varied considerably, yet those from any one tumor are enough alike and enough different from those of any other tumor to be assigned to their own kind. Fibroblasts would also show similar variations. The cells from any one tumor vary like the leaves on a tree, no two are exactly alike, yet a red oak leaf is a red oak leaf, and a maple leaf a maple leaf, in spite of considerable variation in size, shape, etc. You would also note that the cells from the spindle cell sarcomas are more like each other than they are like those from the round cell sarcomas or the polymorphous and mixed cell sarcomas. It is probable that the malignant cells of spindle cell sarcomas come from fibroblasts. It is not so evident from what cells some of the other sarcomas arise.

The general architecture of malignant fibroblasts is similar to that of normal ones and is best seen in cells flattened out on the coverglass. The spread-out normal resting fibroblast has a very delicate transparent cytoplasm. It is often difficult to detect the edge in some places, rather easy in others. The more or less excentric oval nucleus is also clear and transparent; the small nucleoli are the only things to be seen in it. The nuclear membrane is extremely delicate. At the center of the cell is the centriole, not usually detectible in the living cell and rarely seen in photographs. About this center are a few granules and mitochondria, then a little further out are the fat globules and more mitochondria. Scattered granules, fat globules and mitochondria occur in the peripheral ectoplasm and cell processes. The mitochondrial threads tend to be radially arranged about the centriole. This is about the picture one gets of normal living fibroblasts that are spread out on the cover-glass in fresh cultures during the first 24 hours. The whole arrangement becomes emphasized in older cultures. The finely granular central area surrounding the centriole becomes greatly enlarged. About this is a zone of neutral red stainable granules and small vacuoles; about this in turn is usually a heavy zone of fat globules and about the periphery of the cell is the relatively clear ectoplasm. The mitochondria are not confined to any one zone but become more or less radially arranged in reference to the central area and centriole. The nucleus is pushed to one side against the fat globule zone by the enlarged central area.

Malignant sarcoma cells have the same general arrangement. The cells from some sarcomas even in the 24-hour cultures have large central areas, some have heavy fat zones, others very little fat, some have scarcely any neutral red granular zone, others have quite a good one, but it is rarely as prominent as in normal cells. In general the resting malignant fibroblasts as seen in cultures have less transparent and more granular cytoplasm, larger nuclei in proportion to cell size, heavier nuclear membranes and larger and more irregular nucleoli than normal fibroblasts. The arrangement of the fat globules is often a little different. The malignant cell accumulates as a rule fewer and smaller neutral red granules than normal cells. The mitochondria tend to be smaller and more numerous than in normal ones. The nuclei of malignant cells are much more frequently deeply indented on the central side than those of normal ones. This pocket may be quite extensive. A superficial examination of

such a nucleus might lead one to think that the nucleus was highly granular, as the nucleus forms a thick shell like a pushed-in rubber ball, around the pocket filled with granular cytoplasm of the central area. The nucleolar material is sometimes so extensive and broken up into granules that it gives the nucleus a granular appearance, but one can still see plenty of clear nucleoplasm.

Malignant cells are usually larger than normal ones but not always. Owing to the variations in the amount of spreading out on the coverglass about the only safe way to compare cells is to measure ones that have become spherical. The malignant cells of some tumors are fairly uniform in size, while those from others vary enormously, such variations being characteristic for the tumor. There is something very difficult to define but rather characteristic about the manner in which normal and malignant cells spread out on the coverglass that enables one to distinguish at a glance a normal from a malignant cell and the various malignant cells from one another. Malignant cells often have peculiar ruffle pseudopodia somewhat like those on macrophages but different from the wavy edge of normal fibroblasts. They are the organs for pinocytosis, a common habit of malignant fibroblasts. Pinocytosis is a fancy name for cell drinking, a habit normal fibroblasts rarely indulge in. The macrophages, as I pointed out long ago, are great drinkers. Phagocytosis is also more common with malignant fibroblasts than with normal ones. One could go on for some time relating how this and that type of malignant cell differs from a normal one, much to your confusion.

There is no one startling character which serves to distinguish malignant cells from normal ones, yet among those which I have studied in tissue cultures, and they comprise ones from 27 different tumors from the rats and mice, there is no great difficulty in recognizing most of them. The whole cell is more or less altered. It is a sort of a constitutional thing. There are all sorts and combinations of slight differences between normal and malignant cells and between the various types of the latter. Long familiarity, as with many other things in life, enables one to distinguish at a glance differences and qualities that a long and arduous description would fail to reveal.

There are, however, some malignant cells such as those from the spontaneous adenocarcinomas of the mouse that seem to have no visible malignant characteristics, according to Mrs. Lewis and Strong.

It will be noted that the term mutant has not been mentioned for these altered cells. A mutation, according to the geneticists, depends on some alteration in the genes or chromosomal complex. In common parlance mutant may be used for any sort of alteration that descends from generation to generation, but the general inference has been when speaking of malignant cells as mutants, to assume that their peculiarities are due to gene alterations. Up to the present there is no proof that chromosomal or gene alterations are responsible for the various malignant cells and it would be very difficult to prove that the genes were altered even if they were. The chromosomes are numerous and small, and it will be a long long time before one can see in them anything at all comparable to what the giant chromosomes of the salivary gland of the fruit-fly reveal.

Malignant cells are notoriously afflicted with chromosome troubles. The amazing variations in the chromosome picture of fixed and stained preparations in sections and cultures hold the eye to the exclusion of the rest of the cell, which does not show much of anything anyway in such preparations. It is often worse than looking for the soft parts in a fossil.

More mature consideration of such facts as we have at hand has convinced me, temporarily at least, that the chromosome abnormalities are only the manifestation of a more subtle trouble of the cell. A boil, a fever and leucocytosis are manifestations of an infection, not the cause of it. A short survey of the chromosomal abnormalities of malignant cells may convince you also that they are secondary phenomena to other changes in the cell rather than primary, even though one has not determined exactly the primary change.

Most if not all tumors show cells with abnormal chromosomal complexes and mitotic figures as well as normal ones. Some tumors display an astonishing array of abnormal mitotic figures that are repeated in a long line of serial transplanted tumors. There occur cells with the haploid, diploid, tetraploid and greater numbers of chromosomes. In addition cells are encountered with a few more or less than the above numbers. In these tumors all sorts of irregularities of cell division abound, such as division into three or even four equal or unequal cells with an equal or unequal number of chromosomes. Cells with such variable numbers of chromosomes frequently undergo mitoses and are encountered in cultures, especially those from the Walker spindle-cell sarcoma 338 made from time to time over a period of years. This leads one to suspect that the exact full complement of chromosomes or any exact multiple thereof is not essential to the continued life of a malignant cell. One wonders if after differentiation is completed the exact number of chromosomes is essential for the continued life of any somatic cell. In cultures of embryonic chick tissue one occasionally encounters normal cells with abnormal division figures such as tripolars where the chromosomes have every chance of being unevenly distributed to the three daughter cells. I have been told that

abnormal mitosis are sometimes encountered in inflammatory areas.

The unequal distribution of chromosomes during tripolar divisions of malignant sarcoma cells comes about from the usual condition of unequal size of the three limbs of the Y-shaped metaphase plate and also from the fact that total numbers of chromosomes in the three-limbed metaphase plate is usually not sufficient to give each of the three daughter nuclei the normal number of chromosomes. The interesting thing in connection with the variable number of chromosomes in such cells is that the tumor cells from any one tumor are all essentially alike in spite of the fact that some of them are small and have small nuclei and few chromosomes, that some are intermediate and have intermediate sized nuclei and that some are large, have large nuclei (giant nuclei) and more than the normal number of chromosomes. Since otherwise the general cytological and cultural characteristics of such malignant cells of any one tumor are similar to one another and to those with the normal number of chromosomes it would follow that chromosomal variation has no particular effect on the cytoplasm except increasing or decreasing its volume.

Malignant cells with two to several nuclei are frequently encountered in cultures from several of the tumors without noticeable change in the character of the cytoplasm. The nuclei in such cells usually vary in size and often no two nuclei even in the same cell are of the same size. From studies on the tripolar divisions with unequal distribution of chromosomes and cytoplasm to daughter cells we find that the nuclei resulting therefrom vary in size according to the number of chromosomes. It seems probable, therefore, that multinucleated cells having two or more nuclei of unequal size have not two, three or four times the number of chromosomes but variable numbers something more or less than 2, 3 or 4 times the normal number yet the cytoplasmic characteristics remain unchanged. The same thing applies to various sorts of normal cells with two or more nuclei.

Abnormal distribution of chromosomes may also come about through the occurrence of lagging and aberrant chromosomes. Not infrequently one or more chromosomes fail to get into the metaphase plate and when the two groups of chromosomes pass to the daughter nuclei they are not included, but are left in the cytoplasm where they form small chromosomal vesicles. A somewhat similar displacement of chromosomes occurs when one or more chromosomes fail to pass to the daughter nuclei from the metaphase plate. Such lagging chromosomes are left behind, outside the daughter nuclei where they also form small vesicles. The ultimate fate of such chromosome vesicles and cells containing them is unknown, but the latter retain their essential cytological characteristics as long as they have been followed. The cytological characteristics of malignant cells after they have become differentiated are thus apparently not dependent on the maintenance of the exact complement of chromosomes. From this it seems probable that chromosome or gene alterations have nothing to do with the origin of malignancy. The variable distribution of chromosomes in malignant cells is probably secondary to alterations of other parts of the cell, the cytoplasm and centrosomal system.

That chromosome troubles are not primarily due to gene alteration is also borne out by the experiments in our laboratory, on normal cells in cultures, of Mrs. Lewis on the effects of fluorescent X, of Rosenfeld on the effects of ether and ammonia and of Whitman on those of radium.

Fluorescent X causes the terminal ends of some of the chromosomes to adhere so that they fail to completely separate at the usual time during anaphase. This results in lagging chromosomes and unequal distribution to the daughter nuclei. In some cells one or more lagging chromosomes were omitted from each daughter nucleus, in others the two sister chromosomes passed into one daughter nucleus. In strong concentrations of the dye the chromosomes failed to separate into two groups. Cytoplasmic division ensued, however, and the chromosome complex was mechanically squeezed into two masses to form the nuclei for two daughter cells with an unequal distribution of chromosomes.

Rosenfeld found that when normal cells in metaphase or anaphase were subjected to ammonia the progress of mitosis was interrupted. Sometimes the chromosomes became aggregated into a single nucleus with the tetraploid number and on the return to the normal culture medium formed a large resting nucleus normal in appearance in a large cell in which the cytoplasmic division was suppressed. Sometimes after the initial aggregation the chromosomes became scattered in the spindle area and after the return to the normal medium, when cleavage occurred the daughter cells contained unequal numbers of chromosomes. He also found that ether produced abnormal mitoses, following an initial aggregation of the chromosomes. On return to normal medium a variety of events occurred. Sometimes cleavage was suppressed, as with ammonia, and a large nucleus with the tetraploid number of chromosomes or a binucleated cell resulted. Radium also produces abnormal mitoses, lagging and aberrant chromosomes and unequal distribution to the daughter cells, yet radium has never been known to produce tumors in man in spite of extensive use.

I do not care to push too hard at the idea that malignancy is due primarily to cytoplasmic alteration

rather than chromosomal or gene ones, but I am inclined to consider it from that angle at present. The important point which I wish to emphasize is that malignant cells are permanently altered cells that breed true. They are new types or species of cells. This undoubtedly holds for the malignant cells of spontaneous as well as induced tumors. Many spontaneous tumors seem to arise *de novo* as the result of unknown factors at play within the organism entirely unconnected with any outside environmental effects. Others seem to arise from a combination of environmental and autofactors as in locations of chronic irritations. I often wonder if irritable people are more subject to brain tumors than placid ones. If autofactors can produce malignant cells perhaps they can also produce useful alterations. Who knows but that something of this sort has played an important rôle in our evolution and even in our development from the egg. Genes seem to hold the stage just now, but it is not at all clear just how they induce development or evolution. The field is still open to speculation, one of the great sports of mankind.

SCIENTIFIC EVENTS

THE BRITISH NATIONAL PHYSICAL LABORATORY

THE annual report of the National Physical Laboratory, which appeared recently, according to a summary in the London *Times*, states that during 1934, the year under review, there was an increased demand for industrial investigations, which was most marked in the work called for by the shipbuilding industry. The much greater attention given throughout the country to the subject of noise was also reflected in the work of the laboratory, and there was an increase in the number of investigations.

At the William Froude Laboratory no fewer than 60 different designs of ships were tested, this being the highest number since the laboratory was opened in 1911. The modifications in design suggested and carried out by the laboratory have effected large improvement in connection with the resistance of a number of the vessels, and it is estimated that, assuming only one ship of each type was built, that each was steaming for only 200 days a year, and that the life of the ships was 20 years, the net saving to the industry in coal bills alone would be £500,000. Observations made of the height of waves in the Atlantic showed that in a storm they might be up to 25 feet high, rising to 40 feet in a hurricane and that the distance from crest to crest might be about 275 feet.

The subject of noise abatement received much attention in the new acoustics laboratory, and assistance was given to the Ministry of Health in connection with the sound-proof properties of modern walls for use in flats, to the Building Research Board on sound transmission through floors, and to the Ministry of Transport on the limitation of noise from mechanically propelled vehicles.

A new wind tunnel has been designed, and is now in operation, which can be used for studying the behavior of miniature aerofoils, a few inches in length, at a wind speed of 650 miles an hour.

The old British radium standard, which was prepared by the late Mme. Curie in 1913, has been replaced by a new standard consisting of a sample of radium chloride of higher purity. The British radium standard is used for determining, by comparison, the quantities of radium in the needles and other containers used by hospitals.

A section of the report dealing with road research states that special apparatus has been constructed at the laboratory for continuous measurement of impact forces while a vehicle is running along a road. Extensive tests are also being carried out with a heavy six-wheeled lorry running over rough and smooth roads near London, and over obstacles placed on the roads, and on a private road near Oxford impact forces at speeds up to 40 miles an hour are being observed. The results so far obtained show that the maximum impact causing damage to road and vehicle does not necessarily occur at the highest speeds.

Tests made with the object of enabling an aeroplane to fly stalled have led to the trial of a new biplane arrangement in which the upper wings are very much tapered while the lower wings slope considerably so that their tips come close behind the narrow tips of the upper wing. This arrangement was found to be as good as that of a normal biplane as regards performance, and to have a much higher degree of steadiness in stalled flight.

The report, a quarto volume of 260 pages, with 59 illustrations, is obtainable from H. M. Stationery Office for 13s. net.

EDUCATIONAL GEOLOGIC TRIPS IN PENNSYLVANIA

THE Pennsylvania Topographic and Geologic Survey in cooperation with the Pennsylvania Department of Public Instruction has recently inaugurated a plan for conducting geologic field trips for teachers and other interested Pennsylvanians. As a preliminary to the trips, some 2,000 copies of the Survey's bulletin 113, "Pennsylvania Geology Summarized," accompanied by a preliminary announcement of the trips, were distributed in March to high schools, normal

schools, colleges and universities throughout the Commonwealth. This pamphlet summarizes the basic principles of geology and presents a condensed résumé of the geology of Pennsylvania. Following its distribution, return-reply cards were mailed to persons likely to be interested in field trips in two selected regions. It was thought advisable to begin with a limited number of trips until it was determined how the scheme would be received. Three excursions were subsequently held. On May 4, two trips were conducted, the first in the morning and the second in the afternoon, in the region of Chambersburg. These were in charge of R. W. Stone and Dr. Bradford Willard, of the Geologic Survey, and Walter E. Hess, of the Department of Public Instruction. Mr. Hess handled practically all the distributing of the literature and notifying the science teachers in high and normal schools. On May 11 a single trip was conducted by Dr. Willard at Stroudsburg. Mr. Hess again represented the Department of Public Instruction.

Elements of physiography, structural geology, paleontology, stratigraphy, mineralogy and economic geology were discussed on the trips with actual illustrations in the field. An average of 40 attended each of the three trips. The relatively small number made for easier handling of the parties and greater individual attention to questions. On the whole the trips were considered satisfactory both by the persons attending them and those who were instrumental in their planning and execution. Because of their success it is hoped to conduct similar trips in different parts of Pennsylvania in the future.

BRADFORD WILLARD

GRANTS FOR RESEARCH OF THE AMERICAN ACADEMY OF ARTS AND SCIENCES

AT its meeting on April 10, the American Academy of Arts and Sciences announced grants-in-aid from the Permanent Science Fund as follows:

To Frank M. Carpenter, Museum of Comparative Zoology, Cambridge, $350, for expenses in connection with a collecting expedition to Kansas for the purpose of adding material necessary for his work on a "Revision of the Lower Permian Insects of Kansas."

To Tenney L. Davis, Massachusetts Institute of Technology, $300, for technical assistance in the preparation and analysis of certain compounds essential to the completion of his study of the reaction of phosphorus trichloride with cuprous chloride.

To Fred W. Emerson, New Mexico Normal University, Las Vegas, $100, for aid in defraying expenses in connection with a study of the plant associations in the White Sands area near Alamogordo, New Mexico, and in collecting material for the study of palisade cells in desert plants.

To Walter S. Hunter, Clark University, $150, for apparatus, assistance and other expenses to be incurred in investigating the inhibition and disinhibition of conditioned reflexes in human subjects.

To J. W. McBain, Stanford University, $250, for material and equipment to be used in a study of adsorption in the air-water interface of various solutions.

To Arthur S. Graves, Brooklyn Botanic Garden, $250, to help meet expenses in an investigation designed to produce a chestnut resistant to *Endothia parasitica*.

To Professor Robert Weill, of the Faculty of Sciences of the University of Paris, $300, for aid in defraying expenses of a visit to the Bermuda Biological Station to study the nematocysts of Coelenterates.

Applications for grants-in-aid from the Permanent Science Fund of the American Academy of Arts and Sciences will be received by the committee until September 15, 1935, for action at the October meeting of the academy. Applications should be made to Professor E. M. East, *Chairman*, Permanent Science Fund Committee, Bussey Institution, Forest Hills, Boston, Mass.

AWARDS OF LATIN AMERICAN FELLOWSHIPS

THE award of six fellowships of the John Simon Guggenheim Memorial Foundation to Latin American scholars who will come to the United States has been announced.

These fellowships are granted on terms generally similar to those governing the John Simon Guggenheim Memorial fellowships awarded to citizens of the United States. They are awarded to men and women, married or unmarried, without distinction of race, color or creed. The stipends both for Latin America and for the United States are usually $2,000 a year.

At the present time Latin American fellowships are available to citizens of Argentina, Chile, Cuba and Mexico, and also to Porto Ricans. The selection of the fellows whose names are now announced was made by a committee which met in New York, with the advice and assistance of leading scholars and advisory committees in the countries concerned. Two hundred applications were presented this year.

The awards now announced to assist the investigations specified are as follows:

PROFESSOR ALFREDO BAÑOS, JR., professor of theoretical physics, National University of Mexico: Studies of the physical nature of dielectric constant and of the conductivity of dielectrics, at the Massachusetts Institute of Technology.

DR. PEDRO J. BERMÚDEZ HERNÁNDEZ, assistant in zoology and paleontology, University of Havana, Havana, Cuba: Studies of Foraminifera, especially from the paleontological point of view, with the purpose of contributing to a correlation of the Eocene faunas of Cuba

with those of the Gulf Coast of the North American mainland, chiefly at the Cushman Laboratory for Foraminiferal Research at Sharon, Massachusetts.

DR. LUIS HOWELL RIVERO, assistant in anthropology, University of Havana, Havana, Cuba: Continuation of taxonomical and biological studies of West Indian fishes, chiefly at the Museum of Comparative Zoology, Harvard University. The fellowship now awarded to Dr. Rivero is a renewal of a fellowship granted a year ago.

DR. ATILIO MACCHIAVELLO VARAS, chief of the Sanitary Inspection Service of the Northern Sanitary Zone of Chile, Antofagasta, Chile: Continuation of studies in the fields of preventive medicine and public health, with especial reference to typhus, at Harvard University. The grant now made to Dr. Varas will enable him to spend a second year at work on problems of typhus in Chile, in collaboration with Professor Hans Zinsser, of the Harvard Medical School.

DR. TEÓFILO ORTIZ Y RAMÍREZ, professor of clinical medicine, Faculty of Medicine, National University of Mexico, Mexico, D.F.: Clinical studies in the field of cardiac physiology, at Harvard University.

DR. ENRIQUE SAVINO, bacteriologist in the Bacteriological Institute of the National Department of Hygiene, Buenos Aires, Argentina: Studies in the field of public health, with emphasis on epidemiology, chiefly at Harvard University.

The Committee of Selection which met in New York consisted of President Frank Aydelotte, Swarthmore College, *chairman;* Dr. Thomas Barbour, professor of zoology and director of the Museum of Comparative Zoology, Harvard University; Dr. Elmer Drew Merrill, director of the New York Botanical Garden; Dr. Antonio G. Solalinde, professor of Spanish in the University of Wisconsin, and Dr. Richard P. Strong, professor of tropical medicine in the Harvard University Medical School.

SCIENTIFIC NOTES AND NEWS

AMONG the honors awarded on the occasion of the seventieth birthday of King George and the twenty-fifth year of his reign, the Order of Merit was conferred on Sir Frederick Gowland Hopkins, Sir William Dunn professor of biochemistry at the University of Cambridge and president of the Royal Society.

THE trustees of the Humane Society of the Commonwealth of Massachusetts have awarded the gold medal of the society to Dr. George R. Minot and Dr. William Parry Murphy, in recognition of their successful discoveries in the treatment of pernicious anemia. The medals were presented by Charles P. Curtis, president of the society, at the Peter Bent Brigham Hospital on May 23. The awarding of the medal to Drs. Minot and Murphy marks a change in the policy of the society, which for a hundred and fifty years has awarded the medal for heroic rescues, where the life of the rescuer was at stake.

MEMORIAL tablets to Samuel F. B. Morse and to Professor John W. Draper were unveiled at the Washington Square Center of New York University on May 28. The tablets were erected by the State Education Department and the Greenwich Village Historical Society to mark the site of the original New York University building, where Morse sent the first message by electric telegraph and Draper made the first photographic portrait of a living person. The tablets were presented by Catherine Parker Clivette, founder and president of the Greenwich Village Historical Society. Granddaughters of the two inventors, Clara Morse and Dorothy Draper Nye, unveiled the plaques. Chancellor Harry Woodburn Chase made the dedicatory address.

THE honorary degree of doctor of engineering was conferred on May 30 by the South Dakota School of Mines on Dr. Lyman J. Briggs, director of the National Bureau of Standards, who gave the commencement address. Degrees were also conferred on the Honorable George H. Dern, Secretary of War; Dr. Gilbert H. Grosvenor, president of the National Geographic Society; Dr. John Oliver La Gorce, vice-president of the National Geographic Society, and Captain Albert W. Stevens, U. S. Army, leader and scientific observer of the stratosphere flight. The ascent from Rapid City, S. D., of the stratosphere balloon under the auspices of the National Geographic Society and the U. S. Army corps is planned to take place as soon as weather conditions are favorable.

HONORARY degrees were conferred by McGill University on May 30 on Dr. A. S. Eve, retiring professor of physics at the university; on Dr. O. T. Avery, of the Rockefeller Institute for Medical Research; on Adelard Godbout, Quebec Minister of Agriculture, and on Abbé Georges Lemaître, professor of astrophysics at the University of Louvain.

AT the seventy-second annual commencement of Kansas State College on May 27 the honorary degree of doctor of science was conferred on James T. Jardine, chief of the Office of Experiment Stations, U. S. Department of Agriculture. The honorary degree of doctor of engineering was conferred on George W. Wildin, of the class of 1892, consulting engineer, of Pittsburgh, Pa., and on Ernest H. Freeman, of the class of 1895, professor of electrical engineering at the Armour Institute of Technology.

OGLETHORPE UNIVERSITY, Atlanta, Ga., at its com-

mencement exercises held on May 26, conferred the degree of doctor of science on Dr. Annie Jump Cannon, assistant astronomer and curator of astronomical photographs at the Harvard College Observatory, and on Dr. Florence Rena Sabin, member of the Rockefeller Institute for Medical Research.

THE honorary degree of doctor of laws was conferred on Arthur Gibson, Dominion entomologist, by Queen's University, Kingston, Ontario, at the spring convocation.

THE presentation of the Herty Medal for this year was made on May 18 at the Georgia State College for Women to Dr. Francis Perry Dunnington, of Charlottesville, Va., at a dinner given by the Georgia Section of the American Chemical Society. Dr. Dunnington has served as professor of chemistry at the University of Virginia for forty-eight years.

THE Lucien Howe Medal in ophthalmology of the University of Buffalo has been awarded to Dr. Joseph H. Globus, associate neurologist at Mount Sinai Hospital, New York City, and to his associate, Dr. Sidney Silverstone, a member of the house staff, for their work on the diagnostic value of visual defects in brain tumors.

ARTHUR H. YOUNG, vice-president in charge of industrial relations of the United States Steel Corporation, received the Henry Laurence Gantt Medal for his work in industrial relations at a dinner in New York City, on May 24, of the Institute of Management of the American Management Association. Professor Sumner H. Slichter, of Harvard University, was the principal speaker.

THE Lister Medal for 1936, which is awarded in recognition of distinguished contributions to surgical science, has been granted to Sir Robert Muir, professor of pathology in the University of Glasgow, who will deliver the Lister Memorial Lecture in 1936 at the Royal College of Surgeons of England. This is the fifth occasion of the award, which is made by a committee representative of the Royal Society, the Royal College of Surgeons of England, the Royal College of Surgeons in Ireland, the University of Edinburgh and the University of Glasgow. It is now seventy-five years since Lister became professor of surgery in the University of Glasgow.

AT the annual meeting of the Medical Society of London, on May 13, the president, Lord Horder, presented the Fothergillian Gold Medal, awarded by the society every three years, to Sir George Newman, who retired in March from the posts of chief officer of the British Ministry of Health and of the Board of Education.

AT a recent meeting of the Paris Academy of Medicine, Professor Crouzon, of Paris, was elected a fellow, and Professor Johannsen, of Sweden, and Lord Moynihan, of England, were elected non-resident fellows.

AT the recent meeting of the Royal Society of Canada at Hamilton, Ontario, Dr. R. W. Brock, dean of the faculty of applied science at the University of British Columbia, formerly director of the Canadian Geological Survey, was elected *president;* L. J. Burpee, *vice-president* and *honorary secretary;* G. A. Young, *honorary treasurer* and *librarian,* and John Patterson, *honorary editor.*

DR. G. J. HUCKER, chief in research in bacteriology at the New York State Agricultural Experiment Station at Geneva, was elected chairman of the Central New York Branch of the Society of American Bacteriologists at the annual meeting held in Ithaca on May 25.

DR. MERRITT L. FERNALD, for twenty years Fisher professor of natural history at Harvard University, has been appointed curator of the Gray Herbarium. He succeeds Dr. Benjamin Lincoln Robinson, who retires at the close of the present academic year after having served for forty-three years as curator of the herbarium.

HERBERT E. IVES, of Montclair, N. J., for many years in charge of research in television and color-photography at the Bell Telephone Laboratories in New York City, has been appointed honorary fellow for research in color science at the Fogg Art Museum of Harvard University.

DR. JOHN L. BRAY, professor of metallurgy at Purdue University for the last twelve years, has been placed at the head of the School of Chemical Engineering to succeed the late Professor H. C. Peffer.

DR. RUTH MARSHALL will retire in June from the faculty of Rockford College, where she has served as professor of zoology for twenty years. She will be succeeded by Dr. Dorothy Richardson, of Mt. Holyoke College. Miss Marshall plans to continue her work on the taxonomy of the water mites.

AT the London Hospital Professor William Bulloch has resigned from the Goldsmiths' Company's chair of bacteriology and has been succeeded by Dr. S. P. Bedson. A new chair of chemical pathology has also been instituted, to which Dr. J. R. Marrack has been appointed.

DR. H. N. GREEN, at present lecturer in pathology in the University of Cambridge, has been invited to succeed Professor Florey in the chair of pathology at the University of Manchester.

DR. ROBERT M. PETRIE, of the department of astronomy of the University of Michigan, has resigned to accept the position of astronomer at the Dominion Astrophysical Observatory at Victoria, British Columbia.

DR. A. C. SEWARD, master of Downing College, professor of botany, has been appointed to represent the University of Cambridge at the sixth International Botanical Congress, to be held at Amsterdam from September 2 to 7, and Dr. G. H. F. Nuttall, Magdalene College, emeritus professor of biology, will represent the university at the tercentenary of the French Academy from June 17 to 20.

AMONG the delegates from Great Britain present at the centenary celebration of the Royal Observatory of Brussels on May 14 were Professor F. J. M. Stratton, director of the Solar Physics Observatory, Cambridge; L. J. Comrie, superintendent of the British Nautical Almanac Office, Royal Naval College, and J. H. Reynolds, of the Royal Astronomical Society, London.

DEAN CHARLES H. LAWALL, of the Philadelphia College of Pharmacy and Science, will sail on July 3 for Copenhagen to attend a meeting of the Committee upon Uniform Method of Opium Assay, which has been working under the auspices of the Health Committee of the League of Nations since 1931. The chairman of the committee is Dr. L. Van Itallie, of the University of Leiden. Other members are Dr. Yasuhiko Asahina, of Tokio; Dr. H. T. Baggesgaard-Rasmussen, of Copenhagen; Professor R. Eder, of Zurich; Dr. A. Goris, of Paris; Dr. A. W. K. De Jong, of Medan, Netherlands; Professor Erich Knaffl-Lenz, of Vienna, and J. R. Nicholls, of London. Dr. LaWall is representing the U. S. Treasury Department as a pharmaceutical chemist assigned to this special research.

THE Committee on Scientific Research of the American Medical Association has made grants to Dr. Richard L. Crouch, assistant professor of anatomy at the University of Missouri, to promote studies on the connections of the diencephalon in the monkey, and to the American Institute for the Deaf-Blind to be used in aid of research relating to the vibratory sense. This work will be carried on under the direction of Dr. Robert H. Gault, professor of psychology, and Dr. A. C. Ivy, professor of physiology in Northwestern University.

DR. RALPH SAMS HAWKINS, head of the department of agronomy of the College of Agriculture of the University of Arizona, delivered on May 22 the address of the retiring president of the Arizona Chapter of the Society of Sigma Xi, on "Research as an Aid in Regaining Arizona's Domestic Cotton Market."

DR. LOTHAR W. NORDHEIM, visiting professor of theoretical physics at Purdue University, gave a lecture at the University of Oklahoma on May 20 on "The Nature of the Metallic State." He also spoke before the department of physics on "Electron Free Path Phenomena in Metals."

A SIGMA XI lecture before the Brown University Chapter was given by Dr. Edgar Allen, of the department of anatomy, Yale University School of Medicine, on "Recent Advances in the Study of Reproduction."

THE Halley lecture of the University of Oxford was delivered on June 5 by Dr. J. S. Plaskett, director of the Dominion Astrophysical Observatory, Victoria, B. C., Canada. He spoke on "Dimensions and Structure of the Galaxy."

THE American Society of Zoologists will hold its thirty-third annual session at Princeton University on December 30 and 31, 1935, and January 1, 1936. The headquarters will be at the Biological Laboratories in Guyot Hall, and arrangements will be made for accommodations at the Graduate College and the hotels in Princeton. Detailed announcement will be sent to the members later.

THE American Association of Cereal Chemists opened its twenty-first annual meeting at Denver, Colo., for five days, on June 4.

THE conference of Pennsylvania geologists, which convened in Philadelphia on May 31, devoted three days to the study of the crystalline rocks of southeastern Pennsylvania, in order to secure additional information as to their origin, age and relations. Some 200 geologists attended the conference. The field trips were under the leadership of Dr. Edward H. Watson, of Bryn Mawr College, and Dr. Benjamin L. Miller, of Lehigh University. Among those who were expected to take part were Dr. George Ashley, state geologist of Pennsylvania; Dr. F. Bascom, U. S. Geological Survey; Dr. Arthur Bevan, state geologist of Virginia; Dr. Marland Billings, Harvard University; Dr. F. Ward, Lafayette College; Dr. Emmett R. Dunn, Haverford College; Dr. Charles Fettke, Carnegie Institute, Pittsburgh; Dr. Marcus I. Goldman, U. S. Geological Survey; Dr. Hugh D. Miser, U. S. Geological Survey; Dr. Edward Sampson, Princeton University; Dr. P. Tolmachoff, Carnegie Museum, Pittsburgh, and Dr. Herbert P. Woodward, Dana College, Newark, N. J.

The Museum News reports that the Buffalo Museum of Science opened on April 4 the Cabana Hall of Man, a gift of Oliver Cabana, Jr. The opening ceremonies included addresses by Kendall Emerson, executive secretary of the American Public Health Association, and Henry Vaughan, health commissioner of Detroit. The Hall of Man is on the main floor of the museum between the halls of primitive races and of heredity and environment. It is an exposition of the structure and

functioning of the human body. Exhibits included are a life-sized radiograph of a living person, body cross sections in model, a moving skeleton actuated by an electric motor, flanked by a disarticulate skeleton, models showing chest and diaphragmatic breathing, a rubber lung, voice production and reflex action exhibits, and exhibits showing the circulation of the blood, all the heart valves and circulation mechanism being demonstrated in action.

THE Department of Geology and Geography at Smith College has announced its plans for the summer program of work in the Black Hills. The study of the Mississippian-Pennsylvanian contact will be continued, and an effort will be made to determine the geographic range of the five faunal zones which have been differentiated in the Upper Cambrian as a result of field and laboratory studies carried on during the past three years. The western flank of the Black Hills will receive especial attention, and the group's activities, directed by Howard A. Meyerhoff and Robert F. Collins, will extend to the Bear Lodge Mountains, north of Sundance, Wyoming.

THE use of 10,000 acres of cut-over timber land in Livingston parish for use as a laboratory in reforestation work has been extended to the department of forestry at Louisiana State University by the Great Southern Lumber Company, of Bogalusa. The same organization cooperated with the department of forestry of the university in establishing a summer forest camp for students at Bogalusa. About 16,000 trees have already been planted in the area by the farm forestry class, under the direction of Ralph W. Hayes, head of the department.

E. G. REX, New Jersey state supervisor of plant pest control, has announced that the Federal Government is establishing a research unit at Morristown, to fight the Dutch elm disease. New Jersey has already spent $65,000 on the program and the U. S. Department of Agriculture $720,000.

COOPERATING with the Minnesota Department of Conservation, the U. S. Biological Survey has recently established the Talcot Lake Migratory Waterfowl Refuge, in Cottonwood County, Minn., and with funds from the sale of migratory waterfowl hunting stamps the bureau is restoring the area to its former usefulness for wildlife. This project is the first to be financed by the duck-stamp revenues. Sponsored by E. V. Willard, conservation commissioner of Minnesota, the Talcot Lake refuge is regarded by the Biological Survey as an example of the results obtained when conservationists work together. Destroyed by drought, the lake is now being reflooded to provide habitat for waterfowl and fishing for sportsmen. This dual purpose is being accomplished through an agreement whereby the State Department of Conservation will acquire the lands and flowage rights and the Biological Survey will construct a dam in the nearby Des Moines River to restore the lake and to control water levels. Approximately half of the 2,035-acre refuge is being conveyed to the United States for the Biological Survey's use as waterfowl breeding and feeding grounds, including a dry savannah that will be restored to its former marsh condition. The remainder, including most of the lake proper, will be administered by the state for the benefit of fishermen and fur trappers, and in accordance with bureau recommendations regulated fishing and trapping will be allowed throughout the area to such an extent as will not interfere with the primary use of the refuge for water-fowl conservation purposes. The state will assist in patrolling and otherwise protecting the refuge, and the bureau will undertake the biological development of the area and will introduce aquatic plants for waterfowl food and cover.

The British Medical Journal reports that at a luncheon on April 20, held to celebrate the diamond jubilee of the Edinburgh University Chemical Society, Professor James Kendall, of the chair of chemistry at Edinburgh, said that the original foundation of this society went back, not to 1875, but to 1785, so that it was the first chemical society in the world. Examination of the register of students at the university in 1785 had established that out of fifty-nine members of the Chemical Society, fifty-three were students attending Professor Joseph Black's class in chemistry. How long the society survived after 1785 they did not at present know, but they hoped it might be possible to locate a descendant of one of the original members who was in possession of some record of its proceedings and history. It was possible that the Chemical Society of Philadelphia, which claimed to be the first chemical society in the world, might be an offspring of the original Edinburgh Chemical Society, because the University of Pennsylvania had been instituted in 1765, under strong Edinburgh auspices, and the coat of arms of Edinburgh University was still to be seen above the entrance to one of its original buildings.

THE royal research ship *William Scoresby* arrived in London on May 14, after seven months' work in Antarctic waters. This was the fourth visit to the Southern Seas, and was occupied entirely in observing and marking whales, this being the purpose for which she was designed and constructed. It is hoped that this work will cast light on the migrations of the whales of the Antarctic whaling grounds. After final provisioning at Simonstown, South Africa, the *William Scoresby* sailed for the pelagic whaling grounds, and on December 1 the first whale was marked. Soon afterwards the ship met pack ice, and from this time until her return whales in varying numbers were con-

stantly met, sometimes in small numbers, sometimes as many as 200 in one day. The grounds visited were those about Bouvet Island; thence southward towards the ice and along the ice edge eastward to 90 deg., that is, to a position off Queen Mary Land in the Australian Antarctic Territory; then westward to the vicinity of Enderby Land. Between her departure from Capetown and her return to that port at the conclusion of the marking, the *William Scoresby* steamed 17,500 miles and passed 122 days out of sight of land. G. W. Rayner, a member of the *Discovery* scientific staff, was in charge of the operations, with Captain C. R. V. Boothby, R.N.R., in executive command.

THE London correspondent of the *Journal* of the American Medical Association writes: "Again the figures of the registrar general show that as a people the English are growing older as a result of the falling birth and death rates. The birth rate for 1934 was 14.8 per thousand of population. In the last thirty odd years the rate has been halved. This fall is without parallel in the history of this or any other country. The infant death rate in 1934 was 59 per thousand live births; in the quinquennium 1901–1905 it was 138. The general death rate has also been falling steadily. In the quinquennium 1901–1905 it was 16.1 per thousand of population; in 1934 it was 11.8, which was 0.4 above that for 1933, the lowest on record. The increasing aging of the population is shown by the proportion of the persons over the age of 70 years per 10,000 of the total. In 1911 they numbered 297; in 1921, 344; in 1931, 426 and in 1932, 434. The registrar general therefore describes the increase in the number of old people as 'an outstanding feature of our vital statistics.'"

DISCUSSION

THE NEW ACTIVE PRINCIPLE OF ERGOT

RECENT work, revealing the presence in ergot of a water-soluble principle acting very promptly on administration by the mouth, has evidently aroused wide interest. In the issue of SCIENCE for March 29 (*Supplement*, p. 10) a short review was given of the paper[1] published on March 16, in which we described the isolation in crystalline form of the chemical characters and the action of the substance responsible for this effect—a hitherto unknown alkaloid, to which we gave the name "Ergometrine." This had resulted from joint work, on which we had been engaged ever since one of us (Moir) first demonstrated, in 1932, that watery extracts of ergot contained a substance different from any of the principles hitherto known, and acting in this way.[2] Though our work had thus extended over nearly three years, it had been interrupted by the circumstance that Moir accepted, during its progress, a pleasant invitation to visit the United States, where, during a visit of some six months, he had the opportunity of lecturing to American colleagues and demonstrating the method of recording contractions of the puerperal human uterus, which had first revealed the presence of this ergot principle, and which enabled it to be detected and measured in the course of our chemical work. It is now evident that the matter was of such interest to colleagues in Baltimore and Chicago, where the lectures were given, as to stimulate investigators in both centers to independent researches, having the object of identifying the unknown principle. Our own quest for it was resumed on Moir's return to London. This concurrent effort has had the result, in many ways satisfactory, that our recognition of the principle as a new ergot alkaloid has received double confirmation, not only independent but almost simultaneous, from both these centers. The March issue of the *Journal* of the American Pharmaceutical Association (p. 185) contains a paper by M. R. Thompson, of Baltimore, who was probably the first to recognize that the unknown principle had alkaloidal properties. His paper is chiefly concerned with an alkaloidal fraction containing it; and, from the details of the physiological action described, it would appear that this fraction, which he calls "alkaloid X," still contained much alkaloid of the ergotoxine type. In a footnote, however, Thompson reports a later success in crystallizing what was very probably our Ergometrine. The issue of SCIENCE for April 19 (p. 388) publishes a statement entitled "Ergotocin," by M. S. Kharasch and R. R. Legault of Chicago. These authors had apparently not yet seen our paper of March 16, or the abstract of it given by SCIENCE of March 29. In their own earlier paper on the subject, by Davis, Adair, Rogers, Kharasch and Legault, published in the *American Journal of Obstetrics and Gynecology* for February (p. 155), the Chicago group described an impure preparation, having a high activity of the type under discussion, and regarded by them as non-alkaloidal; and they there stated that Eli Lilly and Company had made arrangements to prepare and issue this preparation, and had "given it the trade name 'Ergotocin.'" Again in a footnote, these authors recorded a subsequent success in crystallizing the principle; and it seems clear from the note in SCIENCE (April 19) that they have now recognized that the crystalline principle is alkaloidal and desire to transfer to it the name "Ergotocin." The characters which they attribute to it are those of Ergometrine.

We should like to make it clear that it is far from our intention to engage in a discussion of priority on

[1] *British Medical Journal*, i, 520, 1935.
[2] *British Medical Journal*, i, 1119, 1932.

the basis of note-book records. There can be no doubt that these two American investigations were in progress simultaneously with our own, that they led to the isolation of the same substance independently and that the fact of its crystallization was, in each case, briefly announced at a date not distant from that on which we first described its properties and named it. Our concern is to ensure that the further literature in an important field should not be complicated and confused by a multiplicity of names for the same substance. Our paper, in which the name "Ergometrine" was given to the pure alkaloid, had, in fact, already been published before that by Davis *et al.* came into our hands; but, even if we had seen this paper earlier, we could hardly have felt entitled to consider the scientific adoption, for our alkaloid, of the name Ergotocin, which was there mentioned only as the trade name of an impure and supposedly non-alkaloidal preparation. We hope that, in suggesting to our American colleagues the propriety of adopting "Ergometrine" as the proper, scientific name for the pure alkaloid, we shall not be misunderstood as depreciating the contributions of those who have been working in the same field.

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THE PROTECTION OF WHALES FROM THE DANGER OF CAISSON DISEASE

THE interesting account by Laurie[1] of respiration in the large and active whales of the Antarctic has stimulated discussion of the problems presented by the necessarily peculiar respiratory activity of these animals during prolonged submersion at great depths. As to the limits of their dives there may be some question, but there is no doubt that whales descend to the depth of 100 meters and thereby encounter hydrostatic pressure of about 10 atmospheres. A terrestrial mammal which has been breathing air in a caisson at a pressure greater than 2.4 atmospheres encounters the danger of effervescence of the dissolved nitrogen in the tissues if by ascending rapidly the pressure is reduced more quickly than the blood can transport the released nitrogen to the lungs. The resulting bubbles of gas obstruct circulation and cause caisson disease. Whales do not apparently suffer from caisson disease, and yet their respiratory system is, as far as we can see, typical for mammals.

The whale does not, however, enter a caisson and rebreathe air under pressure. It submerges with one lung volume of air. An estimate of lung volume at 10 per cent. of the body volume can not be far wrong and would start the diving whale with about 8 per cent. of its body volume as nitrogen. Human divers do not suffer from caisson disease until they have been exposed to 2.4 atmospheres absolute pressure,[2] so that the human body can evidently rapidly eliminate the amount of nitrogen contained after saturation at the 1.4 atmospheres extra pressure. In 100 cc of blood and other tissues about 1 cc of nitrogen is dissolved per atmosphere of pressure, and in fat about five times as much.[3] Applying these figures to a whale with 25 per cent. fat[4] at 2.4 atmospheres absolute pressure indicates the ability to eliminate rapidly and with safety 1.4 $(0.75 \times 1 + 0.25 \times 5) = 2.8$ per cent. of its body volume of nitrogen. About one third of the nitrogen in a whale's lungs could be safely dissolved in the tissues and rapidly eliminated.

To introduce one third of the nitrogen of the lungs into the tissues would reduce the amount of gas in the lungs by one quarter, would require an increase in pressure of 2.4 times and would consequently diminish the lung volume to $\frac{1}{2.4} \times \frac{3}{4} = 0.28$ of the normal volume at atmospheric conditions.

The next question is, whether the lungs could be compressed by hydrostatic pressure to the extent of much less than one quarter of their capacity. The lungs of diving animals are not freely open to the exterior as in man. The nasal orifices can be tightly closed, and the bronchioles (in the porpoise) are supplied with contractile tissue which can likewise effectively hold air in the lungs.[5] The thoracic cross section is nearly circular and the intercostal and abdominal musculature is strong. These structures could support great pressure, particularly if there were no movement of the supporting respiratory muscles. But, if the intrathoracic pressure remained low, while the external hydrostatic pressure increased, there would only arise another problem of how to prevent the injection of viscera, blood and lymph into the thorax. It seems likely that the pressure in the lungs would be close to the external hydrostatic pressure, and that most of the nitrogen in the lungs would be forced into solution in the blood and tissues.

The whale's nitrogen capacity has been calculated at about 2 per cent. of the animal's volume per atmosphere pressure. Four atmospheres extra pressure would then cause the solution of all lung gases (provided that total collapse of the lungs occurs) and would still dissolve in the tissues only three times as much nitrogen as the human diver can rapidly eliminate. The human safety

[1] Alec H. Laurie, Discovery Reports, vii, 365, 1933.

[2] L. Hill, "Caisson Sickness and the Physiology of Work in Compressed Air," p. 75. London, 1912.

[3] *Ibid.*, p. 171.

[4] Alec H. Laurie, *loc. cit.*

[5] G. B. Wislocki, *Am. Jour. Anat.*, xliv: 47, 1929.

limit is set as the critical pressure below which caisson disease does not occur, but many men have worked safely and endured rapid decompression at much higher pressures.

It is simple to suggest that the average whale is as capable in this respect as even the exceptional human diver. However, it is perhaps worth completing an argument which has been so often the cause of confusion. The danger of caisson disease specifically occurs when nitrogen diffuses so rapidly from the tissues that the critical pressure for bubble formation is reached in the blood vessels. To protect a man, the rate of diffusion is kept small by slow decompression, which reduces the gradient of nitrogen pressure from tissue to lung. The same result might be accomplished in the whale if the structure or composition of its tissues retards the rapidity with which nitrogen diffuses. For example, it is often suggested that the layer of blubber, with its large nitrogen solubility and meager vascularization, would provide for a slow escape of the nitrogen dissolved at high pressure. Corpulent human divers, however, are especially susceptible to caisson disease.

On the other hand, it is quite reasonable to point out Haldane's view that increasing the rate of the circulation removes the blood from proximity with the source of nitrogen before the critical pressure for bubble formation is attained. It is significant that the whale emerges from a deep dive with an oxygen debt and must maintain an active circulation of blood during the period of recovery. The human diver emerges with no oxygen debt and yet sufficiently fatigued to desire the rest which will further slow his circulation. The whale with an oxygen debt possesses likewise the essential conditions necessary for the specific stimulation of blood flow through the central nervous system,[6] and therefore with the precise conditions which are favorable to the avoidance of nitrogen embolisms in the susceptible central nervous tissue.

In reconsidering the situation in the diving whale, it is apparent that all the nitrogen contained in the lungs will be dissolved at about 4 atmospheres hydrostatic pressure, and that further submersion involves no greater physiological problem. Even to dissolve this amount of nitrogen requires the total collapse of lungs and thorax, a difficult process to reverse. But if it is possible and all the nitrogen is forced into solution, the amount present is still only three times as great as any human diver can safely eliminate. During decompression, the circulation in the whale is bound to be accelerated by the stimulus of its large oxygen debt. I believe that it is a conservative estimate that the whale's circulation would be three times as effective as the human diver's at the time of emergence from a deep dive.

In view of the limited supply of nitrogen and the favorable conditions of the circulation there is no reason why a whale with ordinary mammalian respiratory and cardio-vascular systems should be in danger of caisson disease. Any special characteristics of the whale, such as peculiar amount and distribution of the fat and the retina miriabilia, had better be kept in reserve for the solution of other problems of cetacean physiology.

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[6] W. G. Lennox and E. L. Gibbs, *Jour. Clin. Invest.*, 11: 1155, 1932.

THE HELMHOLTZ-KOENIG CONTROVERSY

IN 1870 Helmholtz published results showing that when two notes are sounded on a siren there are waves set up which will produce resonance in tuned Helmholtz resonators. Koenig repeated the experiment, using tuning forks, and failed to produce vibrations in a third tuning fork which was tuned to the difference tone. It is a well-known fact that when two notes are sounded we hear beats whose pitch is the difference between the two original notes.

The controversy which was argued pro and con for twenty-five or thirty years was: Are these tones which one hears due to a vibration or wave in the air or are they subjective tones? If there are waves in the air which produce vibrations in tuned resonators, strings or forks they are called combinational tones. If these waves do not exist in the air the effects in the ear are called beat notes. It seems that both sides of the controversy agreed as to the above distinction between combinational tones and beat notes. There was no quibbling or haziness about definitions. Present-day writers often use the terms beat notes and combinational tones interchangeably. Others seem to make a distinction between the two terms, but the distinction is a matter of pitch. If the pitch is less than 16 or 30, perhaps, the term beat note is applied. If more than 16 or 30 they are called combinational tones.[1]

All experimenters who used sirens and kindred apparatus were thoroughly convinced that combinational tones were a reality. All those who used tuning forks or piano wires as sources were convinced that there were no waves or combinational tones and that the effects were beat notes. Helmholtz and his followers said Koenig and his followers were wrong. Koenig and his group said that Helmholtz was wrong.

Rucker and Edser,[2] using a siren as sources and a tuning fork as a detector, found combinational tones, but when their sources were tuning forks they say they did not find combinational tones. However, they do

[1] Sutton, SCIENCE, March 8, 1935, p. 255.
[2] *Phil. Mag.*, 39: 341, 1895.

not seem to give much significance to this fact. They are sure there are combinational tones.

Hazel[3] shows that both sides were right, experimentally, and that both sides were wrong, in that they did not recognize that there is an underlying fundamental principle of wave motion in the experiments.

Koenig tried to add two sine waves and found nothing but the two parent waves. Helmholtz modulated one wave or frequency with a second wave and found combination tones. In Koenig's work the equation is, $A_1 \sin \omega_1 t + A_2 \sin \omega_2 t = ?$ Mathematically and experimentally, the only frequencies or waves found are the two original frequencies. In Helmholtz's work we may assume that the output is affected by the air pressure in the common air chamber or that the output is proportional to P sin ωt. When two orifices in the siren are open, the pressure at orifice No. 2, say, is $P_0 + P_1 \sin \omega_1 t$ (the pressure varies in unison with the frequency of orifice No. 1). Then the output is, $(P_0 + P_1 \sin \omega_1 t) \sin \omega_2 t$. Thus we have a "product term." The "product term" is shown by mathematics to be two frequencies, the difference and the sum of the two parent frequencies.

Hazel has shown that in every case when the "product term" is present we find combinational frequencies. These combinational tones or frequencies or waves are real waves which can be detected by tuned apparatus.

With the simple addition of waves these combinational waves are not present, and in the case of sound we have beat notes in our ears. However, if the two frequencies or waves are added through non-linear apparatus we have a "product term," and the combinational waves are found. In the case of sound, since we hear beat notes, the logical conclusion is that our ears are non-linear.

Hazel's work clears up the prevailing hazy conceptions of addition and modulation of waves and shows that the two operations are not the same and that they are fundamentally different.

The case of beat notes is somewhat the reverse of the physiological question: "If a tree falls in the center of a vast forest where there is no animal life, is there any sound?" Physicists will agree that there are waves in the air. There are waves but no ears. With beat notes there are ears but no waves—no air waves whose frequencies are the frequencies of the beat tones heard.

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GONADECTOMY AND A NEW SECONDARY SEXUAL CHARACTER IN FROGS

AN extraordinary and entirely new secondary sexual character in tailless amphibians has recently been described by Dr. C. C. Liu, of Soochow University.[1] This structure, the morphological relationships of which are fully described by its discoverer in a recently published paper,[2] consists of a band of connective tissue extending the entire length of each layer of the Obliquus muscle, at both their dorsal and ventral borders. In certain species at least, the ventral bands are continued inward toward the midline at certain of the inscriptiones tendinae. These bands have been named the *Lineae Masculinae*. As with many other sex-limited modifications in Amphibia, the functional significance of these structures is not immediately apparent. The most obvious assumption—that they are concerned with the mechanics of voice production—is rendered doubtful by their complete absence in many species that are excellent singers. They are found, among other species, in sexually mature males of the common American and European ranids, but are lacking in the bufonids of these regions, and are not found in females of any species. Parker[3] has erroneously stated that they are confined to the two species of *Kaloula, borealis* and *manchuriensis*. Liu has been able to show that they occur in a great many species of the frogs and toads of the world.

It is curious that the presence of a structure as sharply defined as this should have escaped observation until now, particularly in an animal that has been subjected to the minute and continuous scrutiny that has been applied to the frog. Once seen, the lineae masculinae are immediately apparent when a male frog has been skinned. Thousands of students must have observed them unconsciously in American and European laboratories. That they should have escaped the searching eyes of the German anatomists of the last century is still more remarkable. Only the chance combination of a transparent skin and almost complete lack of sexual dimorphism in the Chinese frog *Kaloula borealis* revealed them to Liu. From this starting point he has traced them through the Salientia of the world.

The restriction of the lineae masculinae to one sex suggests a correlation of some kind between them and the gonadal hormones. Their absence in sexually immature animals and an apparent lack of seasonal variation are also significant. As a rule, sexual dimorphism in frogs and toads is not great. The most obvious sex-limited characters are the growths, asperities and glandular accretions which have achieved

[1] C. C. Liu, "Secondary Sex Characters of Chinese Salientia." Thesis, Cornell University, 1934 (abstract, 6 p.).

[2] C. C. Liu, "The 'Linea Masculina,' a New Secondary Sex Character in Salientia." *Jour. Morph. Physiol.*, 57: 131–145, 1935.

[3] H. W. Parker, "A Monograph of the Frogs of the Family Microhylidae," London: British Museum, 1934.

[3] *Phil. Mag.*, p. 103, January, 1935.

such an extraordinary diversity among these animals.[4] In addition, as might be expected, those species which have been intensively studied have been found to show more or less minute sex-correlated differences in nearly every detail of their anatomy. Experimental work on salientian secondary sexual characters has been for the most part confined to those found in easily obtained American and European species of the genera *Rana* and *Bufo*, and the characters which have been available for experimental analysis are singularly uniform and conservative when compared with the bizarre developments seen in many exotic species. With the exception of the vocal sacs, which Champy believes are self-differentiating,[5] and the accessory reproductive apparatus (Müllerian ducts, seminal vesicles), which have long been known to be under the control of the hormones in their post-pubertal development, the characters which have been available for study are seen to be integumentary modifications. Numerous experiments have definitely established that these structures depend upon the sexual hormones, not only for their initial development, but also for their conservation. Occasionally museum collections yield specimens among exotic species that are undergoing sex-reversal, and the condition of the secondary sexual characters in these individuals indicates that the mechanism governing integumentary modifications is uniform throughout the Salientia. While it is impossible to forecast the nature of the supposed correlation between the linea masculina and the gonads, it is immediately apparent that its morphological expression is basically different from the relatively superficial structures represented by nuptial pads and other modifications of the integument. The potential importance, from the sexual standpoint, of the discovery of this sharply defined structure in a standard laboratory type is evident. Scarcely less interest attaches to its functional significance and its curious absence in bufonids and other groups.

In an attempt to determine the nature of the supposed relationship between the linea masculina and the gonads, a number of leopard frogs (*Rana pipiens* Schreber) were gonadectomied during the months of April and May, 1934. An additional series maintained under identical conditions served as controls. The gonads were removed surgically through the customary single abdominal incision, which was sutured with a couple of stitches to prevent prolapse of the viscera. Recovery was rapid and complete, except in those animals that were heavily parasitized or were subjected to undue operative shock. The frogs were maintained in excellent condition by regular feedings of cubed beef liver about every third day. The importance of maintaining experimental animals in a healthy condition, as well as of making a careful examination for regenerated testicular fragments, has been strongly emphasized by Champy (*loc. cit.*). Eight completely castrated males survived operative shock and parasitization. They were killed for observation, along with controls, at irregular intervals between 30 and 163 days. In each case a careful examination was made for indications of regenerated testicular tissue, suspected fragments being subjected to histological examination. Although there was some regeneration of minute nodules of fat, in only one case was a fragment of testis found. Two specimens were retained for 152 and 163 days, respectively, before they were killed. At this time the autopsy revealed that the bands were still fully developed in both operated animals and controls. Histological examination, which shows that the bands are composed of dense white fibrous connective tissue, likewise failed to show any castration effects. Results were also negative in males that had been retained for shorter intervals before they were killed; and the linea failed to develop in a small series of females from which both ovaries had been removed.

The negative nature of these results is not surprising, since the linea masculina obviously forms a part of the basic supporting structure of the body, and experimental work on other vertebrates has shown that many deeply seated somatic differences are not dependant on the sexual hormones for their conservation, once they are fully established. They are of considerable interest in showing the relation between this new secondary sexual character and those sexual characters in frogs that have already been studied so intensively. Two conclusions may be drawn from the results outlined above: (a) Although the testicular hormone may be necessary for the initial development of the linea masculina, it is not necessary for its conservation; and (b) the linea masculina is not potentially present in both sexes in the adult stage, as Zahl and Davis found to be true of the caudal ocellus in *Amia*.[6] Several other possibilities remain. The bands may possibly attain somatic expression entirely independently of the gonadal secretions, as Champy found to be true of the vocal sacs, although this hardly seems likely in view of the apparent time correlation between their appearance and the onset of the testicular hormone. The true nature of the relationship may be revealed by additional castration experiments or by

[4] G. K. Noble, "Biology of the Amphibia," Chap. V, 1931.

[5] C. Champy, "Les caractères sexuels considérés comme phénomènes de développement et dans leurs rapports avec l'hormone sexuelle," Paris, 1924, p. 107.

[6] P. A. Zahl and D. D. Davis, "Effects of Gonadectomy on the Secondary Sexual Characters in the Ganoid Fish *Amia calva* Linnaeus," *Jour. Exper. Zool.*, 63: 291–307, 1932.

heterosexual gonad transplants. Further experiments along these lines are being conducted.

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A CHAT

I HALTED to peruse a piece of modern commercial advertising and was excited by it. It was a neat pamphlet, entitled "———— News Chats," which is periodically published and circulated by one of our huge concerns whose business is the sale of scientific laboratory supplies. It announces itself as being "A bulletin of newsy information to those who know us well and an introduction to those who do not, who we hope will become our friends and customers."

Allow me to reproduce the titles found on some of the articles in the last issue—October, 1934—of this genial monthly visitor from the land of trade to the desks and minds of us lords of American science:

Thar's Sillimanite in Them Thar Hills
A Field Trip in the Classroom
A Hole in a Black Derby Hat
Fitting Trees to the Soil
Black Light from Sunshine
Now Liquids Are Polished
Lots of Agitation for a Little Money
Keeps Storage Batteries Healthy
On the Lookout for J. Pluvius

Furthermore, grant me liberty to quote, with briefest comment, several sentences from these articles—so cleverly and intimately, not to say adroitly, named!

Just being out-of-doors in the bright sunshine is stimulating, but the most excitement comes in searching out interesting insects and animals to see how they *build their homes and raise their children.*

"'Chose qui piait est a demi vendu,' runs the French saying; a thing that pleases is half sold—a truth we all must admit."—To be sure.

"Stranger, that 40 over yonder is the finest hardwood soil you ever want to see."—Yessiree!

"Light, the intangible something that enables us to see things and promotes the growth of plant and animal life, was so much a mystery to ancient mankind as to be deified in some form in almost all of the earlier religions."—Startling information!

"The value lies in the 'eye appeal' which leads prospective buyers to choose one in preference to the other."—I see.

"Only a *healthy,* active storage battery gives its owner a normal period of service."—Honestly?

However, if blame there be for this infantilism in these high places, do not suspect that I lay it upon the publisher. He has a business, and must chat accordingly. This must be a proper approach—effective and profitable—to his audience, else, having tried it, he would not continue it. The great analyzers themselves are analyzed. The business sense has an instinctive power of psychological insight that amounts to wizardry. We are to believe that the "———— News Chats" is a shrewd, welcome and successful adaptation, in the tough realm of competitive trade. Why not?

The average scientist, even the super scientist, of the present day does likewise, as his turn comes to sell, so to speak, his products to his customers—the public and the world. It is the vogue. He cleverly composes his material; cites the business man or else Lewis Carroll as his justification, and then goes out talking folk-lore, even baby dialect, quite naturally and congenially. He acquits himself most adorably before "The Boy Scouts," "The Ladies Better-Fed Club," "The Tradesmen's League Against the Spinning Wheel" and other advanced organizations whose members are simply spoiling for enlightenment in the black magic of all the sciences. Yes, let a second and living Jacques Casanova call to-day upon a second and living Voltaire, and the latter mention some popular modern scientist—as he mentioned on that past day the name of Count Algarotti, the prominent Venetian scientist—and Casanova would be obliged to repeat his famous comment: "That is how he made his name. He constituted himself an admirer of Newton, and made it possible for the ladies to talk learnedly about light."

Though long neglected and out of use, the amazing pedagogical potency of "chat" or the "chatter" method has been rediscovered and re-employed. I am informed that scientific causerie is again very prevalent, even in the erotic wit of the best social circles. They say it is not unusual there, in these days, to hear sweet and burning passion vent itself and forward its cause in language such as the following: "In compound ratio of your affection," "In inverse ratio of my languor," "The mass multiplied by the velocity of my attendance equals the momentum of your passion," "The squares of the times of my hope are as the cubes of the distance of your consent" and so forth—quotations from a French work on Italy, in the second half of the eighteenth century. Sciencized gallantry—what can't science achieve, once it dismounts from its high-horse!

And so, to chat. Just folks, all around; just one big chatting family. Shan't we just sit down now, all together, and enjoy a little chat over a "true black body" or, what amounts to the same, "a hole in a black derby hat hung from a hook on a surface covered with black felt"?

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SCIENTIFIC BOOKS

INEQUALITIES

Inequalities. By G. H. HARDY, J. E. LITTLEWOOD and G. PÓLYA. Cambridge, England, The University Press; New York, The Macmillan Company, 1934; pp. xii + 314. $4.75.

THIS book is devoted to a systematic and critical study of a number of inequalities which are fundamental in mathematical analysis, and to the presentation of a wealth of others which the authors have encountered in their wide experience, many of which have been subjects of their own investigations. It is unique in its field for many reasons, but especially on account of the great variety of results presented and the thoroughness with which the inequalities have been analyzed and generalized. Mathematical investigators will find it an indispensable source of information.

Most mathematicians regard inequalities as auxiliary in character and would perhaps not think of them as constituting a domain of principal interest apart from applications. In reading the book it is a pleasant surprise, therefore, to find that the theory of inequalities is a fascinating subject in itself, and to see how effectively the theory may be systematized and correlated by skilful analysts. The authors have achieved much in this regard, and the results of their efforts indicate the possibility of still further interesting correlations in the future. Their plan is outlined in excellent fashion, with regard to both content and method, in Chapter I, which concludes with some helpful advice to the reader who may be interested in principal results rather than details.

Chapters II–VI contain a systematic theory of generalized arithmetic and geometric means and the relationships between them. The very important inequalities usually designated by the names of Hölder and Minkowski appear as special cases of these relationships. For a finite number of variables the inequalities are treated in Chapter II, for a denumerable infinity of variables in Chapter V, and for functions and integrals in Chapter VI. Chapter III is one of the most interesting in the book. It contains a theory of still further generalized means in which the special function $\phi(x) = x^r$ appearing in the original definition of the authors is replaced by a strictly monotonic function $\phi(x)$. Chapter IX is auxiliary in character, devoted to the explanations of various devices from the calculus useful in deducing inequalities.

In words of the authors "the rest of the book (Chs. VII–X) is written in a different spirit and must be judged by different standards. These chapters contain a series of essays on subjects suggested by the more systematic investigations which precede. In them there is very little attempt at system or completeness. They are intended as an introduction to certain fields of modern research, and we have allowed our personal interests to dominate our choice of topics." Thus Chapter VII is devoted to the proofs of a variety of special integral inequalities which are related primarily by the interesting fact that they can all be established by means of the theory of the calculus of variations.

The material in Chapter VIII has to do with multilinear forms in n sets, each containing a denumerable infinity of variables. For convenience here we may agree that such a set of variables x_i defines a point in a Hölder space if a sum $(\Sigma|x_i|^p)^{1/p}$ with $p > 0$ is finite. The chapter begins with a very general theorem specifying an upper bound for a multilinear form whose variables define points in Hölder spaces related to each other by suitable conditions on the exponents p. The theorem has numerous interesting applications. In the latter part of the chapter bilinear forms with $n = 2$ are more intensively studied. Properties of bounded bilinear forms are deduced; two special bilinear forms of Hilbert are discussed; and a "convexity theorem" of M. Riesz for bilinear forms is developed and applied. Chapter IX is devoted to an important theorem of Hilbert giving an upper bound of the special bilinear form $\Sigma x_i y_k/(i+k)$, with analogues for integrals and with numerous modifications and extensions.

Chapter X contains theorems concerning rearrangements of two or more sets of non-negative numbers, and corresponding theorems concerning rearrangements of functions. A fundamental theorem for two sets $(a_1, \ldots, a_n)$ and $(b_1, \ldots, b_n)$ is that the sum $\Sigma a_i b_i$ is greatest when the notations for the sets are so chosen that the elements of both sets are increasing (or decreasing) in magnitude, and least when their elements vary monotonically in opposite senses. There are similar theorems involving an arbitrary finite number of sets. Departing for a moment from the language of the authors we may define a rearrangement of a function $\phi(x)$ measurable on $0 \leqq x \leqq 1$ as a second function $\bar{\phi}(x)$ such that for every pair of values y_1 and y_2 the measure of the set of points x at which $y_1 \leqq \bar{\phi}(x) < y_2$ is the same as that of the corresponding set for $\phi(x)$. A non-negative function $\phi(x)$ integrable on $0 \leqq x \leqq 1$ has a decreasing rearrangement $\bar{\phi}(x)$ uniquely defined almost everywhere. The theorems concerning rearrangements of finite sets have analogues for functions $\phi(x)$ when sums are replaced

by integrals and monotonic rearrangements of sets by decreasing rearrangements $\overline{\varphi}(x)$.

In this connection a paper by Haskins[1] should be mentioned, which seems to have escaped the attention of the authors. Haskins defined (p. 184) the "momental constants" of a bounded measurable function $f(x)$ on an interval $a \leqq x \leqq b$, which except for a constant factor are somewhat specialized cases of the means $\mathfrak{M}_r(f)$ of Hardy, Littlewood and Pólya. He showed (p. 185) that the values of these constants are characteristic of the class of rearrangements of a function, as defined in the last paragraph above, and describes (p. 194) the increasing rearrangement of a function as typical of the class. Furthermore he proves (p. 189) that the means $\mathfrak{M}_r(f)$ have the effective upper and lower bounds of the function $f(x)$ as limits when r approaches $+\infty$ and $-\infty$, respectively. These results are very closely related to some of those given in the book here reviewed. I understand from Professor Haskins that the paper by Schlömilch, referred to in the book, was not available to him in the war-time year, 1916, when his paper was written. Schlömilch's paper deals with similar conceptions for continuous functions and Riemann integration.

No description of the book here reviewed would be complete without mention of the very valuable lists of theorems and examples at the ends of the chapters. If proofs were given for all these results the book would be expanded beyond reason, but in most cases the necessary arguments are clearly indicated or references are cited. This is only one of many features which insure the great value of the book as a contribution to our modern mathematical literature.

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BIOLOGY FOR EVERYMAN

Biology for Everyman. By SIR J. ARTHUR THOMSON. Two volumes; pp. 1561. New York, E. P. Dutton and Company. 1935.

AMONG the biologists living during the last fifty years, perhaps no one has had such wide and diversified interests as the late J. Arthur Thomson. It must be at least forty years ago that a reviewer, contemplating one of his comprehensive works, expressed doubt concerning the possibility of covering so wide a field. He said that he was quite willing to concede that professors knew more about these matters than any one else, and that of all professors, Scottish professors knew most, and yet, after all, what were the limitations of the human mind? At a later date, it was Thomson himself who, in his charming little book on Herbert Spencer, commented on a result of that philosopher's universality; "we can hardly picture the man who has not some crow to pick with Spencer." So it must be, yet with our scientific babel of tongues, it is a saving grace that there are some, if only a few, who can approximate to a universal language and give us an understanding of the whole drama of life, rather than isolated fragments. In attempting to do this, there are two possible methods. One is to condense and simplify, describing vital phenomena in general terms, but not discussing details. Huxley knew how to do this to perfection. But this synthesis, to be rightly appreciated, must rest on a background of knowledge previously acquired. The other method, followed in the book now reviewed, is to describe details in such a manner as to give a vivid impression of living things in all their diversity, while at the same time constantly recurring to the underlying philosophy which relates them to a whole. The reader is stimulated and delighted to discover how much of interest is going on in this world of nature, indeed, in his own immediate vicinity; so much to observe and enjoy which he has not hitherto noticed. Yet as the Reverend Wm. Kirby, famous pioneer entomologist, said over a hundred years ago, all these things can be seen to illustrate the wisdom and goodness of God. We probably do not express ourselves in theological terms, but it comes to much the same thing if we say that we perceive the harmony and unity of nature, the marvelous creative power which we describe as evolution. So we are alternately, or almost simultaneously, analytic and synthetic, guided by the feeling which Tennyson tried to express in his poem on the "Flower in the Crannied Wall." Sir Arthur Thomson knew well how to set these matters forth in interesting languages for the most part intelligible to any educated person. His book is extremely "readable." But neither Thomson nor any one else can simplify biology in such a way as to excuse the reader from any intellectual effort. In truth, we are dealing with the most complex and marvelous phenomena in the universe and those who have grown old in their investigation still feel like beginners. It is this eternal freshness of biology that constitutes one of its principal charms, for those who care to think.

It is encouraging to note that throughout this country there is an increasing interest in biological subjects, an impetus which, when given sufficient opportunity for development, may carry us far. Thomson, in his concluding chapter, sums up the reasons for being interested in biology, as follows:

(1) Biology can spread our table and increase the amenities of life, ameliorating the struggle for existence.

[1] On the measurable bounds and the distribution of functional values of summable functions, *Transactions* of the American Mathematical Society, vol. 17 (1916), pp. 181–194. See also Jackson, *ibid.*, pp. 178–180; Van Vleck, *ibid.*, vol. 18 (1917), pp. 326–330.

(2) It can conquer disease and help towards an increase of positive health.

(3) It can offer good counsel to help man to meet some of the perennial problems of life.

(4) It has a manifold cultural value.

(5) It affords a basis for eugenics.

(6) It is full of ethical suggestiveness.

(7) It has contributions to make toward a sound philosophy.

Therefore, he says, let us have more biology.

Although the two volumes contain over 1,500 pages, they necessarily leave very much unsaid, and if the book is widely read, as it is certain to be, it should stimulate the production of other works along similar lines. One can imagine books dealing with special groups of animals or plants, or special types of behavior, or with the natural history of particular regions. Also, Thomson's book itself is likely to appear in several editions. It did not get the final revision it might perhaps have had, if the author had lived to see it through the press, and there are naturally some errors to be corrected. It would be tiresome to try to enumerate all these in a review, but one or two may be cited as examples. The reference (p. 1369) to Pleistocene fossil tsetse flies originated in a mere blunder in a very excellent work and has been uncritically quoted. The *Hybernia* moth (p. 869) is cited as a butterfly, evidently because the facts were taken from a German work, which uses the same word for moths and butterflies. The giant cactus (*Carnegiea*) is said (p. 1180) to inhabit Texas. The accounts of fish scales are misleading, not distinguishing between the circuli and annuli. It would be worth while, for the purposes of the next edition, to submit the various chapters to specialists, and so far as possible eliminate these minor errors. They do not much affect the book as a whole, but as they are discovered, they undermine confidence. Furthermore, many of the illustrations could be much improved, and some additional ones would be valuable. The printers and publishers must be congratulated on producing so large a book with hardly any misprints.

T. D. A. COCKERELL

SCIENTIFIC APPARATUS AND LABORATORY METHODS

AN APPARATUS FOR DEMONSTRATING THE OERSTED EFFECT

THE magnetic effect of an electric current is usually demonstrated by bringing a current-carrying wire into the neighborhood of a compass needle, after the manner of Oersted's original experiment. Convection currents, *e.g.*, those carried in electrolytes or in gases, are capable of producing the same effect, but this is not often shown explicitly.

In the belief that it might be advantageous to emphasize the fact that a conduction current in a wire, *i.e.*, one borne by charged particles of one sign moving in one direction, is essentially equivalent in its external magnetic action to electrical convection currents, in which particles of opposite sign move in contrary directions, the following simple apparatus was constructed: A straight wire (W), a long electrolytic cell (E) and a Geissler tube with a straight central capillary (G) were mounted on a wooden base, as shown in Fig. 1. A shallow cylindrical depression under the center of each unit accommodates an ordinary magnetic compass. The electrolytic cell is merely a piece of 8 mm glass tubing bent to the appropriate form, and may be filled with a solution of cupric sulfate. Ordinary copper wires whose ends are twisted into small spirals serve as electrodes. The discharge tube may be any long "I"-tube usually available in the laboratory. Single pole knife switches mounted on the base control the current through the wire and cell, while the electrodes of the Geissler tube are connected directly to the secondary of a small induction coil.

A convenient method of connecting the source of current—a six-volt storage battery—to the remainder of the apparatus is shown in Fig. 2. This arrangement

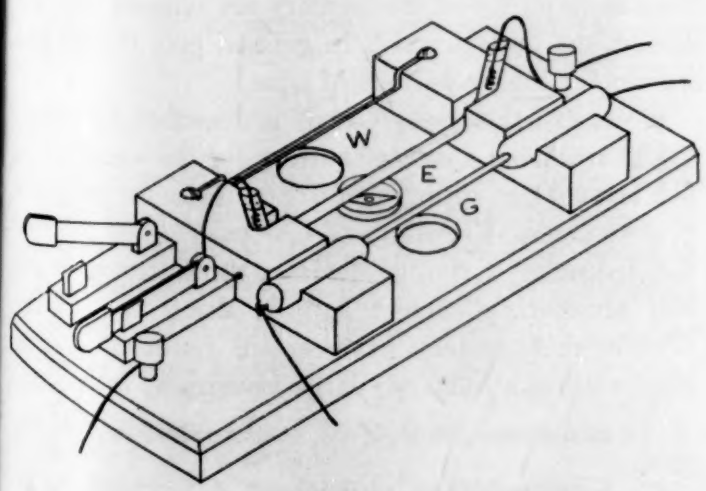

FIG. 1.

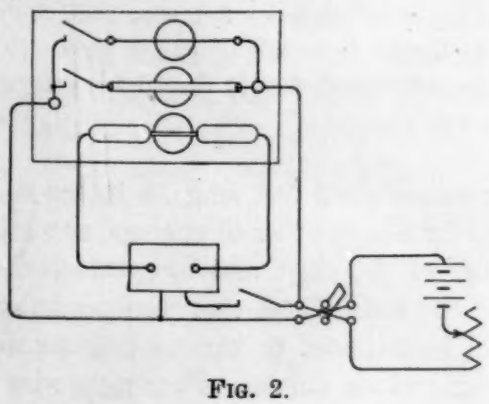

FIG. 2.

makes it possible to demonstrate the three units in rapid succession. One reversing switch serves to change the direction of the current in any unit. An inclined plane mirror clamped above the apparatus makes the effect visible to a large class.

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AN INEXPENSIVE APPARATUS FOR THE MEASUREMENT OF BODILY ACTIVITY

IT is at times important to obtain objective records of the bodily activity of animals without great expense and yet by means of a sensitive instrument. The following apparatus has been used successfully with young puppies and may be adapted to larger or smaller animals.

A small light aluminum baking pan $11'' \times 7'' \times 1\frac{1}{2}''$ (see a in Fig. 1) was suspended within a packing box

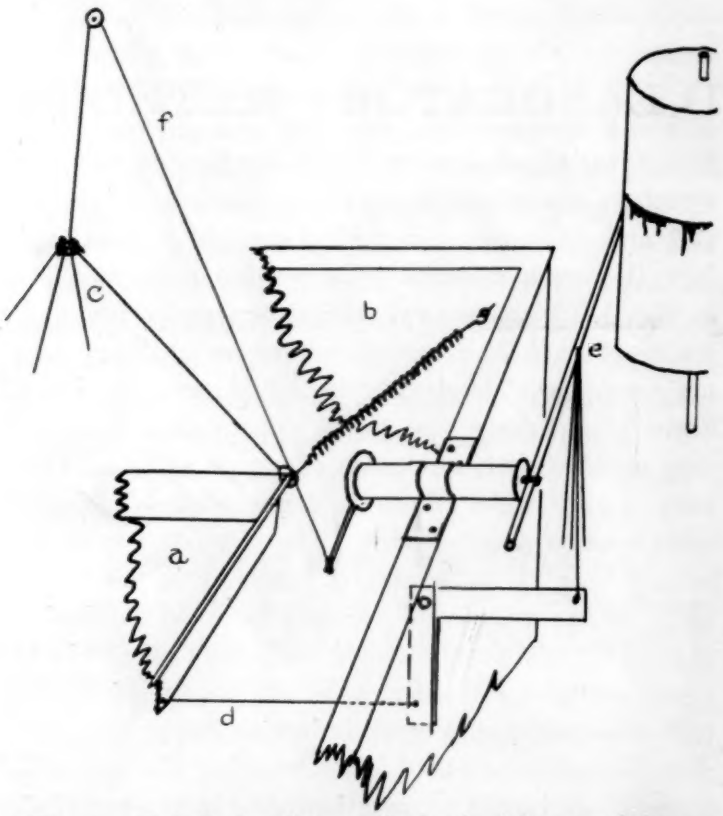

FIG. 1. Detail showing method of wiring used in apparatus for measurement of bodily activity: (a) aluminum pan; (b) packing box; (c) wire rods from corners of pan; (d) thread from side of pan to lever; (e) writing arm with threads attached; (f) thread from upright wires, leading to writing arm.

(b) (approximately 2 feet long, 16 inches wide and 1 foot deep) by means of small springs, one attached at each corner of the tray, and to eyes screwed in the corners of the box. These eyes were so arranged that they could be adjusted to various heights, depending on the weight of the animal. Four light wire rods (c) projected from each corner of the tray to meet above its center.

In order to secure a single record from all movements of the tray, heavy threads (d) were attached to it, one on each side. By means of pulleys these threads converged at a series of levers amplified 3/2, and from the levers threads were connected to a writing arm (e) bolted to a bicycle bearing.

A thread attached to the upright wires from the corners of the tray, which converged above it, was arranged by pulleys in such a way (f) as to pull downward on a lever attached to the bicycle bearing opposite to the writing arm. This lever was bolted so as to make it adjustable to the weight of the animal. Thus, with the tray under slight tension on all sides and with respect to gravity, movement in any direction resulted in a downward pull of the writing arm.

If the animal studied is very active, it may be confined within a ventilated box, which may be placed in the tray, or the box itself may be wired in the way described.

The apparatus has proved sufficiently sensitive in the case of puppies to record practically all movements of skeletal musculature.

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A SENSITIVE A–C VACUUM TUBE RELAY

A VACUUM tube relay possesses numerous advantages in the temperature control of laboratory apparatus which outweigh the slight increase in the complexity of the system. The reduction of the current which passes through the mercury regulator from ten or a hundred milliamperes to the few hundredths of a milliampere required by the vacuum tube practically eliminates all sparking at the mercury contact and makes the presence of moderate amounts of dirt or oxides in the mercury surface a matter of no consequence. This results in a twofold advantage: first, special precautions as to purity of the mercury are unnecessary, and second, the regulator will in general give trouble-free service for longer periods of time.

A vacuum tube relay circuit is described by Rosenbohm[1] requiring a storage battery for the vacuum tube filament current supply and dry batteries for plate and grid voltages. Korpiun and Geldbach[2] show a circuit for operating a similar device with batteries or 220 volt alternating current supply, using two triodes. Both of these systems have certain disadvantages, the first requires a relatively large investment in batteries

[1] E. Rosenbohm, *Proc. Acad. Sci. Amsterdam*, 35: 876, 1932.

[2] J. Korpiun and Alfred Geldbach, *Z. Electrochem.*, 39: 755, 1933.

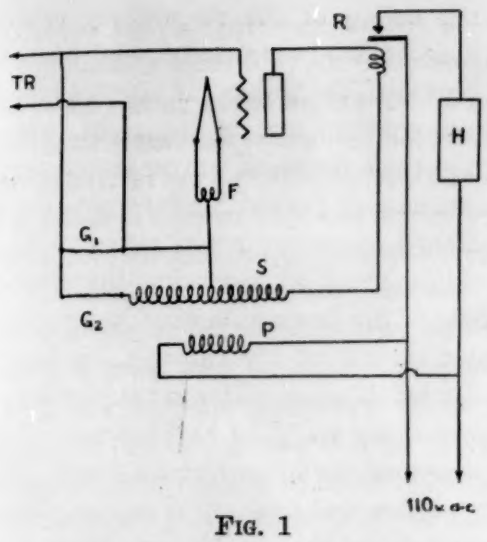

FIG. 1

and occasional interruptions of service for recharging the storage cells, and the second is relatively complex and requires the manufacture or purchase of a number of special resistances.

The author feels that a description of the simple a-c relay developed in this laboratory will be useful to others requiring a sensitive relay for temperature control or other purposes. The circuit is shown in the accompanying diagram. The relay consists of a "45" power amplifier vacuum tube, a Leach No. 1305 a-c relay (R), two resistors (G_1 and G_2) and a transformer with a 110 volt primary (P), a 660 volt center tapped secondary (S) and a 2.5 volt center tapped filament supply winding (F) (Inca transformer, type C-31). TR is the mercury thermoregulator and H is the thermostat heating element. The only electrical supply required is 110 volts a-c. The power consumption (exclusive of H) is 35 to 40 watts. The resistors G_1 and G_2 have the values 2 and 7 megohms, respectively. The parts for the relay are commercially available and inexpensive.

A relay similar to the above has given nearly two years of trouble-free service, regulating the temperature of a covered water thermostat to $\pm$ 0.002° C. and another is controlling the temperature of an open stirred water bath at 25° $\pm$ 0.01° C.

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SPECIAL ARTICLES

ON THE GRAPHIC REPRESENTATION OF IONIC EQUILIBRIA IN BLOOD SERUM

DURING the session of 1915–16 I made a study of ionic equilibria in sea water of 2.3 millimols per liter alkaline reserve, and plotted the results on log-log paper.[1] Log [H⁺] was measured on the x axis and log. CO_2 pressure (later reduced to mm of mercury) on the y axis. During the session of 1916–17 I plotted similar values for blood serums, but in this case the alkaline reserve (bicarbonate), titrated in a rotating hydrogen electrode vessel, varied from sample to sample, so distinguishing marks were used for each sample in marking the values on the log-log paper and it was found that the values of bicarbonate formed a logarithmic scale on an axis at 45° to the x and y axes.[2] Later I learned of the mathematical treatment of this subject by Hasselbalch[3] and applied the equation

$$[H^+] = k_2 \frac{pCO_2}{[BHCO_3]}$$

to that point on each graph where $pCO_2 = [BHCO_3]$ and hence $[H^+] = k_2$ and $pH = pk_2$ (denoting log of reciprocal of k_2). It was found that pk_2 of sea water was 7.08 at 0°, 7.20 at 10°, 7.32 at 20° and 7.44 at 30° at the points where $[BHCO_3] = pCO_2$, but inspection of the graph showed that k_2 varied slightly with CO_2 pressure. Whether this was due to partial change of $BHCO_3$ to B_2CO_3 with fall of CO_2 pressure or due to experimental errors was not determined. In case of blood serums it was thought that errors in titrating $[BHCO_3]$ would be greater at lower values, and with the higher values $pk_2 = 7.5$ at 20°. From the data on sea water it seems evident that pk_2 of blood serum would be at least 0.12 higher at 38° than at 20° and so a value of $pk_2 = 7.62$ might be guessed at. Preliminary attempts at determination of k_2 at 38° showed varying results and were interrupted by my entrance into military service, and after the war I constructed log-log-pH graph paper on three axes at angles of 60° with each other and posted it in the laboratory for the class in physiological chemistry. Since then many papers have appeared on ionic equilibria in blood and new values of the standard hydrogen electrode higher than those used by Sörensen have been used.

Hasselbalch and most later workers instead of titrating $[BHCO_3]$ of serum, added acid and pumped out the CO_2 and measured it and calculated $[BHCO_3]$ and, instead of using pCO_2 in an equation, first calculated the CO_2 physically dissolved in the serum, calling it "H_2CO_3," using two constants k' and c where $k'c = k_2$.

$$[H^+]_s = k'c \frac{pCO_2}{[BHCO_3]_s}.$$[4]

where $c = 0.0591\alpha$.[5]

[1] Publication No. 251, Carnegie Institution of Washington, p. 36, Fig. 6, 1917.

[2] *Jour. Biol. Chem.*, 519: 522, Fig. 1, 1917.

[3] *Biochem. Z.*, 78: 113, 1917.

[4] L. J. Henderson, "Blood," New Haven (1928) equations 6–7, p. 42.

Earlier workers used Bohr's value of $\alpha = 0.541$ at 38° in which case $c = 10^{-1.495}$, whereas most recent workers have used Van Slyke, Sendroy, Hastings and Niel's[6] value of α of 0.51 at 38°, in which case $c = 10^{-1.92}$. Hastings, Sendroy and Van Slyke[7] reviewing recent literature found pk′ averaged 6.104, using Bohr's α, and 6.13, using their own value of α. *In either case* $pk_2 = 7.625$. If, however, the value given in their summary of $pk' = 6.10$ is used, pk_2 becomes 7.62. *Since the value of* α *is not the same for all serums it seems of advantage to use* k_2 *in place of* $k'c$ *and plot quantities that can be directly determined: i.e.,* pH, $p\mathrm{CO}_2$ and $[\mathrm{BHCO}_3]$. Hence I have redrawn my log-log-pH paper with $k_2 = k'c$ of Hastings, Sendroy and Van Slyke, and reproduce it here (Fig. 1).

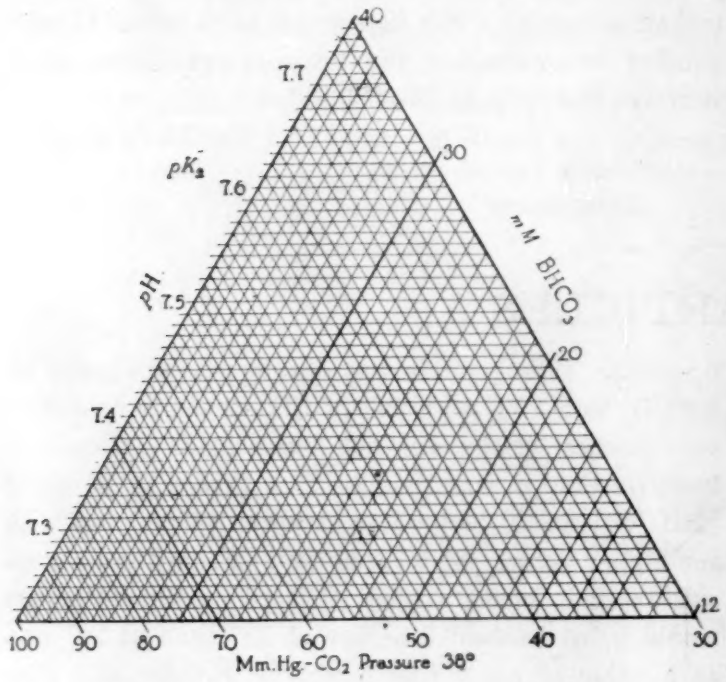

FIG. 1.

Since at all points where $p\mathrm{CO}_2 = [\mathrm{BHCO}_3]$, $p\mathrm{H} = pk_2$, I have marked that value on the pH scale. In case any other value of pk_2 is considered more correct it is only necessary to slide the numbers up or down the pH scale until the pk_2 line corresponds to its new value.

On this graph paper it is easy to mark what takes place during a respiratory cycle or during acidosis or alkalosis, both compensated and uncompensated. In health and comparative rest the values of the blood fluctuate around the center of the chart, being in the arteries above and to the right of the center and in the veins below and to the left of the center. Under extreme conditions the values may go beyond the range of the graph. For example, in order to remove the compensating action of the respiratory center a cat was put under artificial respiration, and when this was markedly increased, the values for arterial blood moved off the graph upward and to the right, whereas the values for the veins remained nearer the center. When the maximum rate of the artificial respiration apparatus was reached an attempt was made to blow more CO_2 out of the blood by removing the surface layer from the base of the lungs with sandpaper and blowing a continuous stream of air through the lungs. The same result was obtained, the venous blood remaining near its normal value. The explanation of this was found in observing the output of the heart. When the respiratory center was put out of action the center or centers controlling the circulation (vasomotor and vagus centers?) regulated the blood and, although the arterial blood was very deficient in CO_2, the blood moved so slowly through the capillaries that its normal CO_2 content was restored.

Since the arterial blood is spread over 125 sq. m. of surface in contact with alveolar air in the lungs, it is safe to assume that the CO_2 partial pressure in the alveolar air is as close to that of arterial blood as could be determined in any ordinary apparatus. Although the partial pressure is not uniform in the different alveoli, the mixed alveolar air should be very close to the mixed arterial blood in CO_2 partial pressure and hence at 38° these three values of pH, CO_2 pressure and bicarbonate concentration may be determined in relation to the arterial blood taken from the living subject with precautions against loss of CO_2 in the sample. In venous blood, however, it seems to me that the CO_2 pressure is the most difficult to determine and it is better to determine pH and $[\mathrm{BHCO}_3]$ and find $p\mathrm{CO}_2$ on the graph.

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SEXUAL PHASES IN PROSOBRANCH MOLLUSKS OF THE GENUS CREPIDULA

PROTANDRY, proterogyny, true hermaphroditism and, occasionally, self-fertilization have long been known to occur in Gastropods. In this group the species of *Crepidula* are of special interest, however, because in *C. plana,* which is normally protandric, it has been thought that the association of the young animal with an older individual, particularly one in the female phase, is essential for the development, as well as for the maintenance, of the functional male phase.[1] In another species, *C. fornicata,* which is likewise protandric, the length of time which the animal spends in the male phase was believed to be correlated with its continued opportunity for insemination.[2]

[5] Peters and Van Slyke, "Quant. Clinical Chem.," 1: 878, equation 11, 1931.

[6] *Jour. Biol. Chem.*, 78: 765, 1928.

[7] *Jour. Biol. Chem.*, 79: 183, 1928.

[1] Harvey N. Gould, *Jour. Exp. Zool.*, 23: 1–69; *Jour. Exp. Zool.*, 23: 225–250, 1917.

In both these species, as well as in *C. convexa,* nearly all individuals pass through a functional male phase while very young, often before the body has attained more than a small fraction of its normal definitive size, as was observed by Conklin many years ago.[3]

The male phase is followed by a series of transition stages, during which the long, muscular copulatory organ and the seminal vesicles are absorbed. Meanwhile the remaining spermatogenic cells of the gonad are cytolyzed, leaving only empty follicles with such ovogonia and ovocytes as were formed in the primary bisexual gonad of the very young animal. In the later transition period proliferation of ovogonia and growth of ovocytes accompany development of the uterus and seminal receptacles characteristic of the functional female phase, as Gould has so fully described.[1]

The two functional sexual phases are thus separated by a more or less extended transition period during which neither sex is dominant, since the animal returns essentially to a state of sexual immaturity. The phases are strictly progressive, however, for the transition gonad invariably develops into an ovary.

In some cases the male phase may be aborted, so that functional sexuality is not realized until the final, female, phase appears. Some individuals, too, show a tendency to remain in the male phase much longer than others. Since this strongly male characteristic is associated with smaller and fewer ovocytes in the gonad, it is thought to be due to a different combination of genetic factors than is present in animals which show a more active and briefer male phase.

This is not strictly a case of so-called "sex reversal"; it is merely the realization of the individual's genetic factors which lead first to the formation of the primary bisexual gonad, then to the functional male phase, followed by the transition stages and terminating in the full sexual maturity of the female phase.

No satisfactory evidence has been obtained to show that this sequence can be altered experimentally, although any one of the phases may be abbreviated or prolonged by various environmental conditions. Nor does it appear that in *C. plana* association of the young animal with older individuals, although usual, is essential for the realization of the functional male phase. Examination of hundreds of young individuals of that species which had attached themselves singly on dead shells of *C. fornicata* showed that such isolated young evidently become as fully functional males as do those which are associated with large females. Both the isolated and the associated young show much individual variability in the size that they reach before assuming the male phase, but the relative sizes are about the same in both environments.

The length of time that the male remains functional is, however, undoubtedly influenced by its environment, as Gould[1] and Orton[2] have observed. This fact is easily proved experimentally for *C. fornicata,* since in this species several individuals pile up in permanently attached groups, usually making a graded series, with the oldest at the bottom and youngest at the top. The oldest has, as a rule, reached the female phase, the younger transition phases and males being superimposed. If these groups be separated and the functional males isolated or segregated, the effects of the changed environment are very striking. Most of the males respond by promptly entering upon the transition stages which lead to the female phase. Spermatogenesis ceases, the penis and seminal vesicles are gradually absorbed and the spermatogenic cells are cytolyzed. Of more than 200 actively functional males which were thus segregated in June, 1934, about 15 per cent. had transformed to the female phase within 63 days; 39 per cent. had reached the third transition stage, 22 per cent. the second transition stage, 12 per cent. the first transition phase, while only about 11 per cent. had remained functionally male. Of an approximately equal number of males of similar sizes which had remained in their normal associations it was estimated that not more than 3 per cent. had reached the female phase during that time, and only 12 per cent. had begun the transition stages, while fully 85 per cent. still retained their function as males.

This experiment might be interpreted as indicating that the females in the intact groups exercise some restraining influence on the normal progressive change of sexuality of the males or that they in some way stimulate the continuation of the male's functions, but at least one other hypothesis should be considered. It must be remembered that each of the males in question has long since become so firmly attached to the shell of the underlying individual that movements are normally limited to merely raising or lowering the shell sufficiently to allow a circulation of the water needed for respiration and nutrition. When dislodged from their normal positions, however, the males struggle vigorously for hours or sometimes for days in efforts to right themselves and secure new attachments. Some of them later resume active locomotion. These active movements not improbably result in a more rapid metabolism which may conceivably initiate the first of the series of interdependent events leading to the sexuality of full maturity. The animal is thus prematurely aged in the sense that the sexual phase normally characteristic of an older age group appears when the body is less than half as large as it might otherwise have been at the beginning of the female phase.

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[2] J. H. Orton, *Proc. Roy. Soc. London,* 81B; 468–484, 1909; *Nature,* 110: 212–214, 1922.

[3] E. G. Conklin, *Jour. Morph.,* 13: 1–226, 1897; *Proc. Acad. Nat. Sci., Philadelphia,* 1898: 435–444, 1898.

Change of sex under these conditions is merely the premature realization of the animal's definitive genetic characteristics.

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PROTECTIVE VACCINATION OF HORSES WITH MODIFIED EQUINE ENCEPHALOMYELITIS VIRUS

By serial passage through pigeons a strain of equine encephalomyelitis virus of the eastern type has been so changed that it promises to be of value as a vaccine. The pigeons were inoculated by the intracerebral route, under ether anesthesia, and the brain tissue for passage secured from birds that had just died or were killed when moribund. The virus has been carried through 100 passages, but most of the work to be reported was done with brains from the 40th and 49th serial passages.

In order to secure more material than is provided by the pigeon brain, a young lamb was inoculated intracerebrally with brain from the 40th pigeon passage and another lamb was likewise inoculated with brain from the 49th passage. Both animals promptly developed encephalomyelitis and died. Their brains were preserved in sterile 50 per cent. glycerin and suspensions were made as needed for the experiments. As little as 1 cc of a 10^{-3} dilution of a 10 per cent. suspension of the brain of either lamb injected subcutaneously into guinea pigs would immunize against from 10,000 to 100,000 infective doses of the unmodified virus injected either subcutaneously or intracerebrally. Of 117 guinea pigs inoculated with the 10 per cent. brain suspension, 8, or 7 per cent., died with symptoms of encephalitis and all but 15 of the remainder were immune. The majority of those that were not immune were tested by intracerebral injection of large amounts of virus. Had they been tested by the subcutaneous route they would probably have lived.

Although the modified virus usually fails to produce disease when injected subcutaneously, if it is brought directly into contact with the central nervous system an encephalomyelitis results. Its activity following intracerebral injection is, however, about 100 times less than that of the unmodified virus. Intracerebral passage of the modified virus through a horse, calf, sheep, rabbit, and serially through five guinea pigs has not restored the lost property of invasion of the central nervous system following subcutaneous injection.

Under controlled laboratory conditions 11 horses have been inoculated subcutaneously with suspensions of the lamb brains mentioned above. The majority of the animals were given 10 cc of a 10 per cent. suspension. Not one horse developed a temperature nor could virus be demonstrated in blood drawn at vario intervals after the injection. With the assistance Dr. J. H. McNeil, state veterinarian for New Jers 67 horses were each given subcutaneous injections 5 cc of the 10 per cent. lamb brain suspension. T inoculations were made in a region where there w many cases of encephalomyelitis, and two of the i oculated animals developed the disease. The vir present in the one brain secured was highly virule for guinea pigs and was evidently not the strain i jected. The other 65 horses showed no reaction to t virus, except that many of those tested as well as those inoculated at the laboratory developed neutr izing antibodies.

Testing the immunity of horses is a difficult proble because the only certain method of producing disea in these animals is by the intracerebral injection virus and only a horse with a very high degree of i munity can withstand such an inoculation. Four o of nine vaccinated animals tested by this meth showed no temperature reaction or other sign of i fection. The other five animals, after an incubati period that was from one to two days longer than t in the controls, developed the disease and died. T other vaccinated horses inoculated intravenously wi virus showed no evidence of disease, but since only o of two controls was infected the results are not co clusive.

In spite of the fact that more than half of our va cinated horses died from a test intracerebral inocul tion, we believe that vaccination with the modified vir will protect against the natural disease. This beli is based on the results of the experiments with guin pigs and on the facts that vaccinated horses develope neutralizing antibodies and that four horses becam so highly immunized that they resisted the intracer bral injection of active virus.

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